Original Article

J Nepal Health Res Counc 2021 Jul-Sep;19(52): 504-7

DOI: https://doi.org/10.33314/jnhrc.v19i3.3421

KRAS Oncogene Mutations in Colorectal Cancer Patients in a Nepalese Tertiary Care Hospital

Bhupendra Kumar Basnet,[1] Balram Gautam,[1] Kamal Ranabhat,[2] Bishnu Dutta Paudel,[1] Sandhya Chapagain Acharya,[1] Sri Ram Bastola,[1] Bishal Gyawali[3]

ABSTRACT

Background: Colorectal cancer is one of the most common cancers in the world and ranks among top ten cancer in Nepal. Limited data have been reported in the literature regarding the prevalence of Kristen Rat Sarcoma viral oncogene mutation in Nepalese patients with colorectal cancer. In a low income country such as Nepal where majority of cancer patient pay for treatment out-of-pocket, it is important to ascertain Kristen Rat Sarcoma viral oncogene mutation status before starting treatment with these agents.

Methods: We analysed 22 colorectal cancer specimens diagnosed histopathologically. Real Time Polymerase Chain Reaction was performed on extracted DNA using RoterGene from Qiagen. US Food and Drug Administration approved kit was used for detection of Kristen Rat Sarcoma viral oncogene mutation i.e. TheraScreen: K-RAS Mutation Kit: The K-RAS Kit detects seven Kristen Rat Sarcoma viral oncogene mutations in codons 12 and 13 of the Kristen Rat Sarcoma viral oncogene.

Results: Kristen Rat Sarcoma viral oncogene mutation was observed in 13 (59%) of the samples studied. All samples had point mutation on codons 12 while 5 samples (38%) also had a point mutation on codons 13. No association was found between the presence of Kristen Rat Sarcoma viral oncogene mutation and gender or age or sidedness of the cancer.

Conclusions: Kristen Rat Sarcoma viral oncogene was commonly present in colorectal cancer specimens. Further efforts towards establishment of diagnostic test, generation of new database, development and scale up of laboratory services are needed throughout the nation.

Keywords: Colorectal cancer; KRAS oncogene; mutation analysis; Nepal; risk factors for CRC

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers in the world and ranks among top ten cancer in Nepal.[1] Kristen Rat Sarcoma viral oncogene (*KRAS)* mutations are considered important predictive and prognostic biomarker in CRC since identification of KRAS mutation is associated with lack of response with Epidermal Growth Factor Receptor (EGFR) monoclonal antibodies.[2]

The prevalence of KRAS mutation in CRC patients is 35-40 %.[3, 4] The three RAS genes (KRAS, NRAS and HRAS) have been implicated in the pathogenesis of multiple cancers, with KRAS being the most common mutation in patients with colorectal cancer.[5]

Geographical differences in *KRAS* mutation have been reported in the literature.[6, 7] Limited data has been reported so far regarding the prevalence of KRAS mutation in Nepali patients with CRC.[8] Since majority of patients pay for treatment out-of-pocket in Nepal, it is important to ascertain *KRAS* status before starting treatment. [9, 10] Our objective was to quantify the prevalence of KRAS mutation in our population and describe the characteristics of Nepalese patients with KRAS positive advanced CRC.

METHODS

This prospective observational study was conducted in the Department of Gastroenterology, the Department of Clinical Oncology and Medical Genetic Laboratory of National Academy of Medical Sciences (NAMS), Bir hospital, Kathmandu, Nepal. Bir hospital is the oldest hospital of Nepal and one of the key central hospitals under the government of Nepal. NAMS is the only academic institution in the country that runs a fellowship program in Medical Oncology. This study was conducted as a part of the establishment of a new

Correspondence: Kamal Ranabhat, Ministry of Health and Population Kathmandu, Nepal. Email: kamal_raj7@iom.edu.np, Phone: +9779851158392.

genetic laboratory at Bir Hospital; we tried to evaluate the efficiency of the newly built genetic laboratory in our center by studying the prevalence of KRAS mutation in our population.

Formalin Fixed Paraffin Embedded (FFPE) tissue samples were initially collected from patients with a histopathologically confirmed diagnosis of colorectal cancer. The samples were collected from October 2017 to April, 2018. Patients had to be over the age of 16 and able to provide informed written consent for inclusion.

Twenty two colorectal cancer specimens were studied. Genomic DNA was extracted from Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections from histologically confirmed primary colorectal tumors using QIAamp DNA FFPE Tissue Kits(Lot number: therascreen KRAS RGQ PCR Kit (cat: 870021). Histopathologically diagnosed colorectal cancer tissue were selected for study. 10% neutral buffered formalin was used for fixation of tumor tissue for such tests. Pathologist selected the tumor tissue for testing. Genomic DNA was extracted from Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections from histologically confirmed primary colorectal tumors using QIAamp DNA FFPE Tissue Kits using manufacturer's instruction. Genomic DNA was quantified using therascreen KRAS RGQ PCR Kit. The sequences of codons 12 and 13 of the *KRAS oncog*ene were genotyped by Real Time Polymerase Chain Reaction (RT-PCR) amplification and direct sequencing according to the validated method. RT- PCR was performed on extracted DNA using RotorGene (Qiagen, Germany). United States Food and Drug Administration (US-FDA) approved kit was used for the detection of KRAS mutation (TheraScreen: K-RAS Mutation Kit). The K-RAS Kit detects seven KRAS mutations in codons 12 and 13 of the KRAS oncogene. We used Fisher exact test to observe the association between KRAS mutation and different variables (age, gender, residence, ethnicity and sites of cancer). To avoid duplication of the sample, we received the samples with mentioning clear patent's ID and name of the patient. Of the total 22 specimens 17 samples were taken from biopsy. Test was validated using positive and negative control provided in therascreen KRAS RGQ PCR Kit in each batch. The genomic DNA extracted was quantified before preforming RT PCR. Normal colorectal tissue was not used to detect the wild type KRAS. Positive and negative control were used in each batch of testing. Internal control was used in each sample to ensure quality control of each test.

This study was approved by the ethical review committee of NAMS. Ethical approval for this study was provided by the Institutional Review Committee of National Academy for Medical Sciences (NAMS), Kathmandu, Nepal (Ref: 499/2077/78). A written informed consent was obtained from the individual participants. Confidentiality and anonymity of the participants were ensured by coding the interviews. Study participants were informed clearly about their freedom to opt out of the study at any point of time without justifying for doing so.

RESULTS

Paraffin embedded samples (n=22) of colectomy or colonic biopsies were studied. *KRAS* oncogene mutation was observed in 13 (59%) of the samples studied, of which 7 (53%) were female (Table 2). Median age observed was 35 years (IQR: 26 to 80). All of the samples had point mutation on codons 12 while 38% (n=5) samples also had a point mutation on codons 13. The most common mutation observed was ASP which was seen in 100% samples. The G>A transition on codons 12 and 13 was seen in 100% cases (Table 3). Single nucleotide mutation was seen in 6 (47%) samples, of which 4 had two mutations and 3 had three mutations. Multiple mutations were more common in females (71%) than males (33%). No association was found between the presence of KRAS mutation and gender or age or sidedness of the cancer (right versus left) (p<0.0.5).

Table 1. Socio-demographic characteristics of study participants (n=22).

Variables	Number of colorectal cancer present, n (%)
Median age (IQR)	
Age (years)	
30-40	10 (45.5)
41-50	5 (22.7)
>50	7 (31.8)
Sex	
Male	11 (50.0)
Female	11 (50.0)
Residence	
Urban	12 (54.5)
Rural	10 (45.5)
Ethnicity	
Brahmin/Chhetri	7 (31.8)
Aadibashi/Janajati	10 (45.5)
Madhesi	5 (22.7)
Total	22 (100)

Table 2. Characteristics features of KRAS mutation positive & negative cases (n=22).

Character-istics	Cases with KRAS mutation (n =13)	Cases without KRAS mutation (n = 9)	Total (n= 22)
Right-sided colorectal cancers	6 (54.5)	5 (45.5)	11 (100)
Left-sided colorectal cancers	7 (63.6)	4 (36.4)	11 (100)
Female gender	7	6	13
Median age (IQR)	45.6 years	44.5 years	

KARS= Kristen Rat Sarcoma viral oncogene

Table 3. Distribution of different KRAS mutations in the CRC patients (n=13).

Mutations	Frequency (%)
CODONS 12	
ASP	13 (100)
SER	2 (15.4)
VAL	2 (15.4)
AVA	1 (7.7)
CODONS 13	
ASP	5 (38.4)

CRC= Colorectal Cancer, KRAS= Kristen Rat Sarcoma viral oncogene

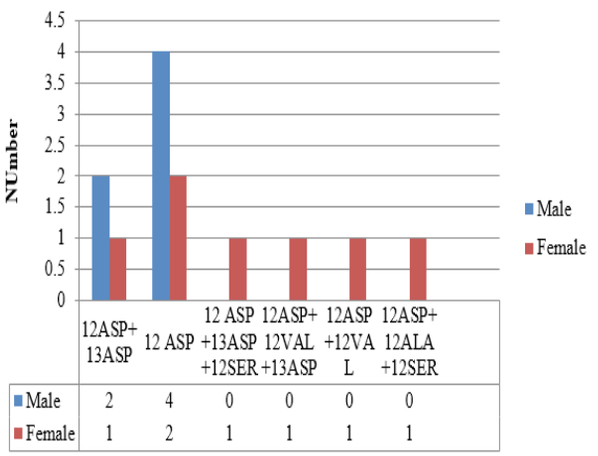

Figure 1. Distribution of KRAS mutation by gender (n = 22).

DISCUSSION

Our study is the first to document the presence of KRAS mutations among patients with colorectal cancer in Nepal. Despite the established role of KRAS mutations as a prognostic and predictive marker in the treatment of metastatic colorectal cancer, KRAS testing has not been available in Nepal until now. This study shows high prevalence of KRAS mutations among patients with colorectal cancer in Nepal paving the way for the establishment of KRAS testing at the Genetic Laboratories in Nepal. However, further work is needed to establish the role of testing other RAS mutations, and determine the cost-effectiveness of using anti-EGFR antibodies based on these tests in the context of Nepal.

Although limited by a very small sample size, we found KRAS mutation in 59% samples. The *KRAS* mutation frequencies in Asian, European, and Latin American mCRC patients have been reported to be 24.0%, 36.0% and 40.0%, respectively.[10, 11] Although our sample size is small to claim a higher prevalence in Nepali cancer patients, it is worth noting that KRAS mutations are not rare among our patients and testing for these mutations may be informative for patients and physicians in Nepal. Previous small studies from Nepal has also revealed prevalence of KRAS mutations in Nepali patients, although not as high as seen in our study but these differences could simply be a function of small sample size.[8] Furthermore, the prevalence of KRAS mutation in our neighboring country, India differs widely among studies with some reporting as low as 19%[8] to as high as 43%.[12] We need more robust study with bigger sample size to establish the true prevalence of KRAS muta tion in our context. To that end, we need to establish whether a cheaper Next Generation Sequencing (NGS) panel could be more cost-effective in testing multiple relevant mutations than to establish testing platforms for each mutation in each tumor type separately.

Our study has also established ASP to be the commonest type of KRAS mutation in Nepali patients, consistent with other reported literature.[10,13-15] However, we were unable to note whether the mutations are more common based on gender or age phenotype. Our study was also limited since we did not have information on treatment received by these patients and whether these mutations affected treatment decisions.

CONCLUSIONS

Kristen Rat Sarcoma viral oncogene was commonly present in colorectal cancer specimens of Nepalese Populations.

ACKNOWLEDGEMENTS

The authors would like to acknowledge all the study

participants who were involved in the data collection process. We would like to express our heartfelt gratitude to the Nepal Health Research Council for providing financial support. We'd also like to thank Mr. Kiran Paudel, Institute of Medicine, Tribhuvan University for supporting data management.

Author Affiliations

[1]Bir Hospital, National Academy of Medical Sciences (NAMS), Nepal

[2]Ministry of Health and Population (MoHP), Kathmandu, Nepal

[3]Queen's University, Kingston, Canada

Competing interests: None declared

REFERENCES

1. Cancer Incidence and Mortality in Kathmandu Valley (Kathmandu, Lalitpur and Bhaktapur Districts). First Year Report of the Population Based Cancer Registry, 2018. [Download PDF]
2. Wilson PM, Labonte MJ, Lenz HJ. Molecular markers in the treatment of metastatic colorectal cancer. Cancer J. 2010 May-Jun;16(3):262-72.[Article]
3. Rosty C, Young JP, Walsh MD, Clendenning M, Walters RJ, Pearson S, et al. Colorectal carcinomas with KRAS mutation are associated with distinctive morphological and molecular features. Modern Pathology. 2013 Jun;26(6):825-34.[Article]
4. Payandeh M, Amirifard N, Sadeghi M, Shazad B, Farshchian N, Sadeghi E, Dayani M. The prevalence of KRAS mutation in colorectal cancer patients in Iranian population: A systematic review and meta-analysis study. Biomedical Research and Therapy. 2017 Oct 16;4(10):1693-704. [Article]
5. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. *J Cell Sci*. 2016;129(7):1287-1292.[Article]
6. Timar J, Kashofer K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. Cancer and Metastasis Reviews. 2020 Dec;39(4):1029-38. [Article]
7. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, et al. The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. British journal of cancer. 2004 Jul;91(2):355-8.[Article]
8. Barman PK, Aryal G, Maharjan S, Sah B, Pandey S, Bhattarai S, Joshi I. BRAF Mutated Metastatic Colorectal Carcinoma (mCRC): A case report. Nepal Mediciti Medical Journal. 2020 Dec 1;1(1):17-20.[Article]
9. Rodenhuis S, Slebos RJ, Boot AJ, Evers SG, Mooi WJ, Wagenaar SS, et al. Incidence and possible clinical significance of K-ras oncogene activation in adenocarcinoma of the human lung. Cancer Res. 1988 Oct 15; 48(20):5738-41.[Article]
10. Inoue Y, Saigusa S, Iwata T, Okugawa Y, Toiyama Y, Tanaka K, et al. The prognostic value of KRAS mutations in patients with colorectal cancer. Oncol Rep. 2012 Nov; 28(5):1579-84. doi: 10.3892/or.2012.1974 21.
11. Smitha CS, Suresh BM, Linu JA, Lakshmaiah KC, Govind BK, Lokanatha D, Pretesh RK. Patterns and the occurrence of KRAS mutations in metastatic colorectal cancers—a study from Indian Regional Cancer Centre. Indian journal of surgical oncology. 2017 Dec;8(4):511-3.[Article]
12. Apte M, Vyakaranam L, Prakash D. Anti-Competitive Agreements In The Indian Civil Aviation Sector-A Need For Reforms. Journal of Critical Reviews. 2019;6(6):297-302.[Download PDF]
13. Behl AS, Goddard KA, Flottemesch TJ, Veenstra D, Meenan RT, Lin JS, et al. Cost-effectiveness analysis of screening for KRAS and BRAF mutations in metastatic colorectal cancer. J Natl Cancer Inst. 2012 Dec 5; 104(23):1785-95. [Article]
14. Veldore VH, Rao MR, Prabhudesai SA, Tejaswi R, Kakara S, Pattanayak S, et al. Prevalence of KRAS mutations in metastatic colorectal cancer: A retrospective observational study from India. Indian J Cancer. 2014 Oct-Dec; 51(4):531-7. doi: 10.4103/0019-509X.175371. PMID: 26842186.
15. Aissi S, Buisine MP, Zerimech F, Kourda N, Moussa A, Manai M, et al. KRAS mutations in colorectal cancer from Tunisia: relationships with clinicopathologic variables and data on TP53 mutations and microsatellite instability. Molecular biology reports. 2013 Nov 1;40(11):6107-12. [Article]