RESEARCH ARTICLE

Editorial Process: Submission:11/07/2023 Acceptance:10/11/2024

Associations of *KRAS* Mutations and Clinical Characteristics of Colorectal Cancer Patients in Indonesia

Reno Rudiman[1], Ahmad N. Alfarisy[2], Kiki Lukman[1], Prapanca Nugraha[1], Yoopie Setiawan[3], Yunia Sribudiani[4,5]*

Abstract

Background: Colorectal cancer (CRC) remains a major burden worldwide, ranking third and second in incidence and mortality respectively. Detection of biomarkers including *KRAS* mutations can help predict prognosis and response to therapy in CRC, thus this study evaluated the frequency of *KRAS* mutations among Indonesian patients and their associations with clinicopathologic characteristics. **Methods:** Fifty-three CRC samples were collected from January to September 2022 in the Department of Surgery, Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. *KRAS* mutations were analyzed using PCR followed by Sanger sequencing. Associations between *KRAS* mutations were evaluated using binary logistic regression analysis. **Result:** *KRAS* mutations were detected in 52.8% of patients (n=28), of which 3.6% were p.Gly12Ser (n=1), 32.1% were p.Gly12Asp (n=9), 7.1% were p.Gly13Asp (n=2), 3.6% were p.Gln61His (n=1), 3.6% were p.Asp126His (n=1), and 3.6% were p.Lys169Glu (n=1). The p.Asp73= polymorphism was detected in 57.1% of the samples (n=16). *KRAS* mutation status did not differ significantly between the groups based on the age of onset, sex, tumor location, tumor histology, stage, and family history. **Conclusion:** *KRAS* mutations are present in high frequency in this cohort of CRC patients in a tertiary hospital in West Java, Indonesia. However, *KRAS* mutation status is not associated with the age of onset, sex, tumor location, tumor histology, stage, and family history.

Keywords: Colorectal Cancer- Early Onset- *KRAS*- Late Onset

Asian Pac J Cancer Prev, **25 (10)**, 3457-3461

Introduction

Approximately one in every ten cancer cases and deaths is due to colorectal cancer (CRC). With more than 1.9 million cases, CRC is ranked the third most common cancer worldwide and is the second deadliest cancer with 935,000 deaths [1]. The incidence increases as socioeconomic status increases and the age of diagnosis is steadily decreasing, attributable to the shift towards a westernized lifestyle among rapidly developing countries [2]. Some high-income countries such as the USA, France, and Germany show decreasing trends of CRC in older patients in the last few decades. This is mainly attributed to the elimination of CRC risk factors including healthier life style, smoking cessation, increased screening, and removal of early lesions [3]. These favorable trends, however, overshadow the increasing trends of early-onset CRC (EOCRC) in both high and low-income countries.

In Indonesia, CRC is the fourth most common cancer but Indonesian CRC patients show distinctive characteristics compared to patients from other regions. A high proportion (20-30%) of Indonesian CRC cases are early onset (EOCRC) [4, 5], nearly triple that in European countries (10%) [6].

From molecular pathological perspective, EOCRC is associated with lower defective DNA mismatch repair abnormalities and/or Lynch syndrome, suggesting a similar sporadic pathway to LOCRC [7]. In sporadic CRC, *KRAS* mutation is the most common somatic mutation. In *EOCRC, KRAS, BRAF V600, NRAS*, and APC are found in lower prevalence compared to LOCRC although some data are conflicting [8]. *KRAS* has five exons, four coding and one non-coding. Forty percent of CRCs harbor *KRAS* mutations, most of which are missense mutations in codons 12, 13, and 61 [9]. *KRAS* mutations are more commonly found in females, proximal tumors, mucinous adenocarcinoma, and more advanced stages [10, 11]. *KRAS* association with poorer prognosis has been controversial with studies reporting that the specific mutations p.Gly13Asp and p.Gly12Cys are associated with poorer overall survival (OS), while mutations in p.Gly12Asp and p.Gly12Val are not associated with

*[1]Division of Digestive Surgery, Department of Surgery, Dr. Hasan Sadikin General Hospital, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. [2]Undergraduate School, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. [3]Master of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. [4]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. [5]Research Center of Medical Genetics, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. *For Correspondence: y.sribudiani@unpad.ac.id*

poorer OS [12, 13].

Considering the unique characteristics of Indonesian CRC patients, additional study on Indonesian CRCs may benefits on drawing conclusions on this topic. This study aimed to evaluate the clinicopathologic and molecular features especially *KRAS* mutation status in the unique Indonesian CRC patients.

Materials and Methods

Patient selection

From 72 samples available for this analytical cross-sectional study, 53 met the inclusion criteria of histopathologically proven CRC at The Department of Surgery, Dr. Hasan Sadikin General Hospital, adequate DNA sample, and complete registry data. The patient samples were evaluated for *KRAS* mutation in exons 2, 3, 4, and 5. The demographic (age, gender) and clinicopathological features (family history, tumor location, histological type, and staging) were obtained from the CRC Registry of the Faculty of Medicine Universitas Padjadjaran and were evaluated for the association with the molecular results. This study was approved by the Research Ethics Committee of Dr. Hasan Sadikin General Hospital (No. LB.02.01/X.6.5/122/2022).

DNA isolation

Tissue samples were collected from colonoscopy biopsy or surgical resection, some of which were immediately sent to the Pathology Anatomy laboratory for Hematoxylin-Eosin (HE) staining and DNA isolation was performed using the Quick-DNATM Miniprep kit (Zymo Research, CA, USA).

Genotyping

KRAS primers were designed using Primer3® and are shown in Table S1. PCR was performed in a total volume of 50 µl containing: 50-100 ng of DNA, 1 µlof 10 µM forward and reverse primers, and 25 µl of 2xMyTaqTM HS Red Mix (Bioline). *KRAS* mutations were detected by touch-down PCR (Td-PCR) with an annealing temperature ranging from 60°C to 50°C. Amplification was confirmed by DNA electrophoresis using 5 µl of PCR product on a 1.5 % agarose gel. The remaining sample was used for Sanger sequencing. DNA amplification in pre-sequencing was performed using a Big Dye Terminator V3.3 kit (Applied Biosystem, Foster City, CA, USA) on an ABI XL 3500 automated sequencer and the sequencing results were analyzed using BioEdit© v 7.2 (Informer Technologies, Inc.).

Data analysis

All variables are presented as categorical variables. Chi-squared analysis was performed in SPSS (Chicago, IL, USA) to evaluate the associations between the categorical data of clinical characteristics and the binomial output of *KRAS* mutations status, with $p<0.05$ considered statistically significant.

Results

Patients Baseline Characteristics

Of the 53 patients enrolled in this study, 19 were males (35.8%) and 34 were females (64.2%) with a median and mean age of 56 (25-80) and 56.64+2.88 years, respectively. The patients were then classified into two groups: EOCRC (<50 years old) and late-onset CRC (LOCRC) (>50 years old). The clinical features of the patients are presented in Table 1

Identified KRAS Mutations

28 patients (52.8%) test positive for *KRAS* mutations, of which 10 (35.7%) were EOCRC and 18 (64.3%) were LOCRC (Table 2). Of all *KRAS* positive patients, 12 (42.9%) harbored the mutation in exon 2, 1 (3.6%) in exon 3, 1 (3.6%) in exon 4, and 1 (3.6%) in exon 5. Polymorphisms were detected in 16 patients (57.1%), all in exon 5. The specific point mutations were: p.Gly12Ser in 1 patient (3.6%), p.Gly12Asp in 9 patients (32.1%), p.Gly13Asp in 2 patients (7.1%), p.Gln61His in 1 patient (3.6%), p.Asp126His in 1 patient (3.6%), p.Lys169Glu in 1 patient (3.6%), and the p.Asp73= polymorphism was detected in 16 patients (57.1%). Sanger sequencing results of p.Gly12Asp and p.Gly12Ser are shown in Figure 1.

Associations of KRAS Mutations and Clinical Characteristics

The association of *KRAS* mutation status and clinicopathologic features i.e., age, sex, family history of cancer, tumor location, stage, and tumor histology were evaluated and presented in Table 3. Although the

Table 1. Clinicopathologic Characteristics of Patients (n=53)

Clinicopathologic Features	N	%
Age		
Early-onset CRC (<50 y.o.)	14	26.42
Late-onset CRC (>50 y.o.)	39	73.58
Sex		
Male	19	35.85
Female	34	64.15
Tumor Location		
Right-sided	12	22.64
Left-sided	41	77.36
Tumor Histology		
Mucinous	3	5.66
Non-mucinous	50	94.34
Stage		
I	1	1.89
II	11	20.75
III	16	30.19
IV	25	47.17
Family History (n=38)		
First degree	2	5.26
Second degree	6	15.79
No	30	78.95

Table 2. Identified *KRAS* Mutations and Polymorphism

No.	Sample No.	Sex	Age	Tumor Location	Nucleotide Change	Amino Acid Change
1	S0004	M	42	Left	c.34G>A	p.Gly12Ser
2	S0007	F	69	Left	c.35G>A	p.Gly12Asp
					c.519T>C	p.Asp173=
3	S0008	F	71	Right	c.183A>C	p.Gln61His
4	S0011	F	50	Left	c.505A>G	p.Lys169Glu
5	S0016	M	46	Right	c.35G>A	p.Gly12Asp
6	S0018	F	56	Left		
7	S0023	F	53	Left		
8	S0027	M	62	Left	c.519T>C	p.Asp173=
9	S0029	F	40	Right	c.38G>A	p.Gly13Asp
					c.519T>C	p.Asp173=
10	S0031	M	68	Left	c.376G>C	p.Asp126His
11	S0034	M	70	Left	c.519T>C	p.Asp173=
12	S0037	M	55	Left		
13	S0040	F	57	Right		
14	S0043	M	68	Left		
15	S0044	F	50	Left		
16	S0045	M	25	Left		
17	S0046	F	57	Left		
18	S0047	F	67	Left		
19	S0048	F	68	Left	c.35G>A	p.Gly12Asp
					c.519T>C	p.Asp173=
20	S0049	F	49	Left	c.35G>A	p.Gly12Asp
21	S0054	F	43	Right	c.519T>C	p.Asp173=
22	S0057	M	46	Left		
23	S0058	M	67	Left	c.35G>A	p.Gly12Asp
24	S0059	F	69	Left	c.38G>A	p.Gly13Asp
25	S0062	F	42	Left	c.35G>A	p.Gly12Asp
26	S0066	F	67	Right	c.519T>C	p.Asp173=
27	S0070	F	46	Left		
28	S0071	F	64	Left	c.35G>A	p.Gly12Asp

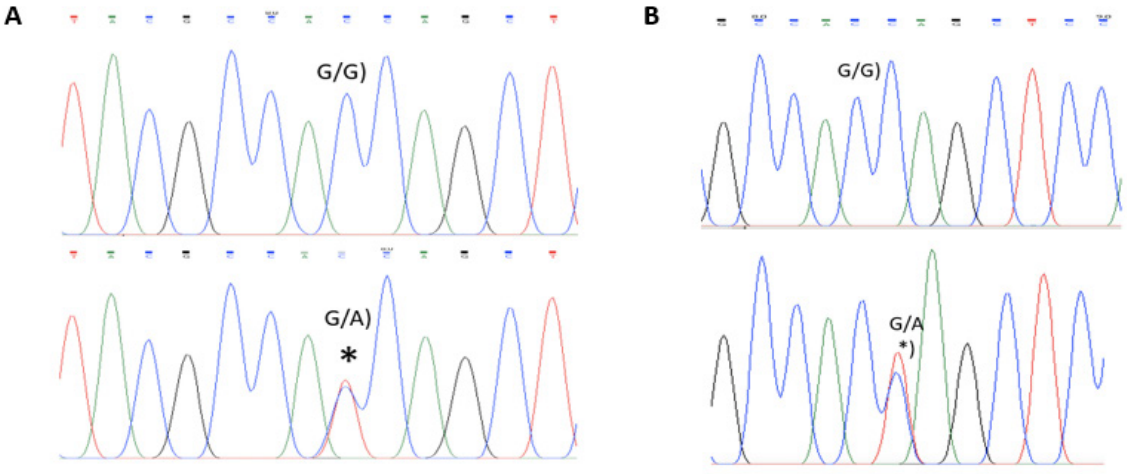

Figure 1. Electropherogram of *KRAS* Mutations. (A) (top) wild-type (bottom) KRAS mutations of c.35G>A/p.Gly12Asp. (B) (top) wild-type (bottom) mutations of c.34G>A/p.Gly12Ser.

Table 3. Association of Clinicopathologic Features with *KRAS* Mutation Status

Clinicopathologic Features	KRAS Mutation, N (%)		p-value
	Yes	No	
Age of Onset			
Early-onset (<50 y.o.)	9 (16.9%)	5 (9.4%)	0.184
Late-onset (>50 y.o.)	19 (35.8%)	20 (37.7%)	
Sex			
Male	10 (18.9%)	9 (17.0%)	0.48
Female	18 (34.0%)	16 (30.2%)	
Tumor Location			
Right-sided	6 (11.3%)	6 (11.3%)	0.648
Left-sided	22 (41.5%)	19 (35.8%)	
Tumor PA			
Mucinous	1 (1.9%)	2 (3.8%)	0.304
Adenocarcinoma	27 (50.9%)	23 (43.4%)	
Stage			
I	0 (0.0%)	1 (1.9%)	
II	4 (7.5%)	7 (13.2%)	
III	9 (17.0%)	7 (13.2%)	
IV	15 (28.3%)	10 (18.9%)	0.502
Family History			
Yes			
First degree	2 (5.3%)	0 (0.0%)	0.955
Second degree	2 (5.3%)	4 (10.5%)	
No	12 (31.6%)	18 (47.4%)	

frequency of *KRAS* mutation was higher in late-onset compared to that in early-onset, however it was no significantly difference groups (p=0.184). There was also no difference between male and female patients. Although the *KRAS*-mutated group had a higher proportion of positive family history, it was not significantly associated with *KRAS* mutation status (p=0.955). *KRAS* mutations were present in higher frequencies in patients with more advanced tumors i.e., stage III and IV (p=0.502), with a higher proportion in left-sided tumors (53.7% vs. 50%) (p=0.648) and fewer in mucinous tumors (54.0% vs. 33.3%) (p=0.304).

Discussion

Similar to the current worldwide cancer burden, CRC is one of the most common cancers in Indonesia. It is the fourth most frequent cancer causing 17,786 deaths or nearly 8% of the total cancer deaths in Indonesia [1]. Furthermore, Indonesian CRC patients show unique characteristics e.g., a higher proportion of younger patients. Therefore, to decrease CRC incidence and deaths, as well as to improve screening and management strategies, several tumor markers have been proposed and used to predict the outcome of CRC patients, including *KRAS* mutations. This study evaluated *KRAS* mutations in 53 Indonesian CRC patients and how they associate with patients' clinicopathologic features.

KRAS mutations were detected in 52.8% of patients, of which, the most common mutations were p.Gly12Asp (32.1%) and p.Gly13Asp (7.1%), similar to previous studies reporting a high frequency of *KRAS* mutations in Romania (45.3%), a Nordic population (52.6%), a Chinese population (37.6%), and in an Indonesian population in the Jakarta region (63.6%) [14–17]. However, However, Abdullah et al. [18] reported a lower frequency of *KRAS* mutations (16.3%) in Indonesian CRC patients in Jakarta. The rare *KRAS* mutation subtypes of p.Gln61His, p.Asp126His, and p.Lys169Glu detected in the present study have not been studied in-depth so more research is required on these Indonesian *KRAS* mutation subtypes and their roles in CRC.

Next, we evaluated the association of *KRAS* mutations with the clinicopathologic characteristics of the CRC patients namely age of onset, sex, family history, tumor location, stage, and tumor histology. Ozer and Goksu [19] found an association between *KRAS* mutations and older age of diagnosis, whereas Watson et al. [7] reported a significantly higher proportion of *KRAS* mutations in younger patients. In Indonesia, to the best of our knowledge, this is the first study to evaluate the association of *KRAS* mutations and age of onset of CRC. In the present study, *KRAS* mutations were not associated with the age of onset but we observed a notably high proportion of EOCRC patients (26.4%) compared to previous European studies (about 10%) [6]. This is supported by previous Indonesian studies by Susanti et al. and Anthonysami et al. which reported proportions of 22% and 31% of young patients respectively [4, 5]. Thus, our study suggests a distinctive characteristic of Indonesian EOCRC patients. A higher proportion of *KRAS* mutations occurred in females, patients with a first-degree family history of cancer, left-sided tumors, more advanced stage, and non-mucinous tumors, although none of these reached statistical significance. Won et al. [10] also showed associations between *KRAS* mutations in females, right-sided tumors, more advanced stage, and mucinous adenocarcinoma. However, Rimbert et al. [20] found that common *KRAS* mutations (codon 2) are associated with classical adenocarcinoma compared to non-RAS mutated patients, while rare *KRAS* mutations (exon 3 and 4) are associated with mucinous adenocarcinoma compared to other *KRAS* mutations. This might explain the higher proportions of *KRAS* mutations in non-mucinous tumors in our study considering the low frequency of *KRAS* mutations in exons 3 and 4.

In summary, *KRAS* mutations occurred in 52.8% of the fifty-three Indonesian CRCs in West Java. *KRAS* mutations were not significantly associated with the age of onset, sex, family history, tumor location, stage, and tumor histology. These findings provide the basis for improving CRC screening and treatment, as well as guide future CRC research.

Author Contribution Statement

RR contributes to study design, diagnosis, sample collection, data analysis and manuscript reviewing; ANA contributes to data collection, laboratory works, data analysis and manuscript writing; KL contributes to study design, diagnosis, sample collection and review

the manuscript; PN contributes to sample collection, data analysis, review the manuscript, data registry; YS (Yoopie Setiawan) contributes to data analysis; YS (Yunia Sribudiani) contributes to study design, data analysis, manuscript writing and reviewing.

Acknowledgements

The authors widely acknowledge and thank the patients and their families for their participation in this study. This is study is funded by Universitas Padjadjaran under The Internal Research Grant RKDU No. 2203/UN6.3.1/PT.00/2022.

Funding Statement

This study was supported and funded by Universitas Padjadjaran under The Internal Research Grant RKDU No. 2203/UN6.3.1/PT.00/2022 for Reno Rudiman, Kiki Lukman and Yunia Sribudiani.

Approval

This study was reviewed and approved by The Research Ethics Committee of Dr. Hasan Sadikin General Hospital (No. LB.02.01/X.6.5/122/2022).

Data availability

All the data that supports this study are included in the manuscript and the supplementary materials.

Conflict of Interest

The authors do not have any conflicts of interest to declare.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.
2. States u, cancer n. United states national cancer database , 2004 – 2015. 2020;125(21):3828–35.
3. Keum N, Giovannucci E. Global burden of colorectal cancer: Emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol. 2019;16(12):713-32. https://doi.org/10.1038/s41575-019-0189-8.
4. Susanti S, Wibowo S, Akbariani G, Yoshuantari N, Heriyanto DS, Ridwanuloh AM, et al. Molecular analysis of colorectal cancers suggests a high frequency of lynch syndrome in indonesia. Cancers (Basel). 2021;13(24). https://doi.org/10.3390/cancers13246245.
5. Anthonysamy MA, Maker LPII, Gotra IM, Saputra H. Prevalence of colorectal carcinoma based on microscopic type, sex, age and anatomical location in sanglah general hospital. Intisari Sains Medis. 2020;11:272.
6. Vuik FE, Nieuwenburg SA, Bardou M, Lansdorp-Vogelaar I, Dinis-Ribeiro M, Bento MJ, et al. Increasing incidence of colorectal cancer in young adults in europe over the last 25 years. Gut. 2019;68(10):1820-6. https://doi.org/10.1136/gutjnl-2018-317592.
7. Watson R, Liu TC, Ruzinova MB. High frequency of kras mutation in early onset colorectal adenocarcinoma: Implications for pathogenesis. Hum Pathol. 2016;56:163-70. https://doi.org/10.1016/j.humpath.2016.06.010.
8. Saraiva MR, Rosa I, Claro I. Early-onset colorectal cancer: A review of current knowledge. World J Gastroenterol. 2023;29(8):1289-303. https://doi.org/10.3748/wjg.v29.i8.1289.
9. Meng M, Zhong K, Jiang T, Liu Z, Kwan HY, Su T. The current understanding on the impact of kras on colorectal cancer. Biomed Pharmacother. 2021;140:111717. https://doi.org/10.1016/j.biopha.2021.111717.
10. Won DD, Lee JI, Lee IK, Oh ST, Jung ES, Lee SH. The prognostic significance of kras and braf mutation status in korean colorectal cancer patients. BMC Cancer. 2017;17(1):403. https://doi.org/10.1186/s12885-017-3381-7.
11. Abadi za, asl dh, rahmani b, peimani a, dodaran ms, rad fs. The clinicopathologic features of colorectal adenocarcinoma with respect to mismatch repair genes and kras status: A retrospective study. 2021;1–11.
12. Modest DP, Ricard I, Heinemann V, Hegewisch-Becker S, Schmiegel W, Porschen R, et al. Outcome according to kras-, nras- and braf-mutation as well as kras mutation variants: Pooled analysis of five randomized trials in metastatic colorectal cancer by the aio colorectal cancer study group. Ann Oncol. 2016;27(9):1746-53. https://doi.org/10.1093/annonc/mdw261.
13. Jones RP, Sutton PA, Evans JP, Clifford R, McAvoy A, Lewis J, et al. Specific mutations in kras codon 12 are associated with worse overall survival in patients with advanced and recurrent colorectal cancer. Br J Cancer. 2017;116(7):923-9. https://doi.org/10.1038/bjc.2017.37.
14. Dinu D, Dobre M, Panaitescu E, Bîrlă R, Iosif C, Hoara P, et al. Prognostic significance of kras gene mutations in colorectal cancer--preliminary study. J Med Life. 2014;7(4):581-7.
15. Yuan Y, Liu Y, Wu Y, Zhang J, Shen C, Zhang F, et al. Clinical characteristics and prognostic value of the kras mutation in chinese colorectal cancer patients. Int J Biol Markers. 2021;36(2):33-9. https://doi.org/10.1177/17246008211017152.
16. Osterlund E, Ristimäki A, Kytölä S, Kuopio T, Heervä E, Muhonen T, et al. Kras-g12c mutation in one real-life and three population-based nordic cohorts of metastatic colorectal cancer. Front Oncol. 2022;12:826073. https://doi.org/10.3389/fonc.2022.826073.
17. Marbun VMG, Erlina L, Lalisang TJM. Genomic landscape of pathogenic mutation of apc, kras, tp53, pik3ca, and mlh1 in indonesian colorectal cancer. PLoS One. 2022;17(6):e0267090. https://doi.org/10.1371/journal.pone.0267090.
18. Abdullah M, Sudoyo AW, Utomo AR, Fauzi A, Rani AA. Molecular profile of colorectal cancer in Indonesia: Is there another pathway? Gastroenterol Hepatol from Bed to Bench. 2012;5(2):71–8.
19. Ozer M, Goksu SY. Age-dependent prognostic value of KRAS mutation in metastatic colorectal cancer. Futur Oncol. 2021;17(35):4883–93. https://doi.org/10.2217/fon-2021-0650
20. Rimbert J, Tachon G, Junca A, Villalva C, Karayan-Tapon L, Tougeron D. Association between clinicopathological characteristics and RAS mutation in colorectal cancer. Mod Pathol. 2018;31(3):517–26. https://doi.org/10.1038/modpathol.2017.119.