RESEARCH ON *KRAS* MUTATIONS IN PATIENTS WITH COLORECTAL POLYPS LARGER THAN 10 MM

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Summary

Objectives: Determine the prevalence of *KRAS* mutations using RNA samples and the association with endoscopic and histopathological images of colorectal polyps larger than 10mm. ***Subjects and methods:*** A cross-sectional study on 84 patients at the Gastroenterology-Hepatobiliary center - Bach Mai Hospital from 01/2017 - 12/2021. Perform a colonoscopy, select the largest polyp over 10mm in size to characterize and perform polypectomy, taking the specimen for histopathology according to WHO criteria 2010. Identification of *KRAS* gene mutations in tissue samples using RNA samples. ***Results:*** 10.7% of the *KRAS* gene is mutated. *KRAS* gene mutation rates tended to be higher in villous polyps compared to tubular polyps (33.3% vs. 9.2%) and high-grade dysplastic polyps compared to low-grade dysplastic polyps (23.1% vs. 8.6%), though no correlation between *KRAS* gene mutations and endoscopic imaging characteristics of polyps has been reported. ***Conclusion:*** *KRAS* gene mutations are not common in polyps larger than 10 mm but are related to the villious component and the degree of dysplasia on the histopathology of the polyp.

** Keywords: Colorectal polyps; Endosco; Histopathology; KRAS mutation.*

INTRODUCTION

The enlargement of the mucosa and submucosa tissues is the primary cause of colorectal polyps, a disease of the digestive system [1]. This condition is fairly typical among gastrointestinal illnesses in general and colorectal illnesses in particular. It is also thought to be a precursor to colorectal cancer.

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Numerous variables influence the progression of polyps to cancer, but the accumulation of *KRAS* mutations is particularly crucial for promoting polyp growth, histopathological villi development, and high-grade dysplasia [2]. Therefore, earlier diagnosis of *KRAS* gene alterations in colorectal polyps, especially those larger than 10 mm improves patient management and prognosis [3].

Since the approach of identifying gene mutations by RNA from biopsy tissue samples has not been used frequently, research to find *KRAS* gene mutations in patients with colorectal polyps has not been documented in Vietnam yet. Therefore, we conducted this study: *To determine the rate of KRAS mutations using RNA samples and the relationship with endoscopic and histopathological images of colorectal polyps larger than 10 mm.*

SUBJECTS AND METHODS

1. Subjects

Consisted of 84 patients diagnosed with larger 10mm colorectal polyps through flexible endoscopy and histopathology at the Gastroenterology-Hepatobiliary center - Bach Mai Hospital from January 2017 to December 2021.

Excluded from the study were patients with polyps associated with colorectal cancer, the prepared colon was not clean for adequate evaluation, there were no polyps larger than 10 mm or histopathology results, *KRAS* mutations could not establish.

2. Methods

* *Study design:* A cross-sectional study.

All eligible patients selected for the study were thoroughly interviewed about their medical history, clinical examination, and performed colonoscopies with the polypoid biopsy.

Colonoscopy has been done on Evis EXERA II CV170, CV180 machine with soft colonoscopy at the Gastroenterology-Hepatobiliary center, Bach Mai Hospital.

We record the number of polyps detected on the endoscopy, then select the polyps with the largest size and over 10 mm to characterize polyps on the following issues:

- Polyp site: Described in 8 colorectal anatomical positions, then united into 2 locations:

+ Proximal colon: Including the cecum, ascending colon, hepatic angular colon, and transverse colon.

+ Distal colon: Including angular spleen colon, descending colon, sigmoid colon, and rectum.

- Polyp shape: Described according to the Paris classification (2005) consisting of pedunculate, semi-pedunculate, and sessile [4].

- Polyp size: divided into 2 levels of 10 - 20 mm and larger 20 mm.

Perform polypectomy (by the snare or EMR method), then take the entire polyp for histopathology test at the Department of Pathology - Bach Mai Hospital. Histopathology results are agreed upon by at least 2 experienced pathologists.

The 2010 WHO classification of polyps includes polypoid and non-polypoid [5].

+ Evaluation of the degree of dysplasia includes low-grade dysplasia and high-grade dysplasia [5].

- *KRAS* mutation detection test: Determination of *KRAS* mutation by Semi-Nested Multiplex RT-qPCR method with RNA extracted from a biopsy tissue sample in patients with a colorectal polyp in Department of Gene Technology and Genetics, Military Medical Research Institute, Vietnam Military Medical University, The candle molding tissue was cut into cross-sectional with a thickness of about 10 μm and transferred to polypropylene Eppendorf. Samples were stored a 4^{o}C until a sufficient number of samples will carry out the process of identifying *KRAS* mutations in the following steps: RNA extraction from samples, reverse transcription and enrichment, excess primer processing, and product analysis with qPCR.

* *Data processing and analysis:* Using SPSS 20.0 medical statistics software. Statistical analysis using the method of calculating frequency, percentage, mean, χ^2, or Fisher exact test. The percentage values are taken 1 digit after the decimal number. The difference is considered statistically significant when the p-value < 0.05.

RESULTS

3. Characteristics of age, gender, colonoscopic images, and histopathology

** Age and gender characteristics:*

Table 1: Age and gender characteristics of research.

Age, gender		Number (n = 84)	Percentage (%)
Age group	< 20	4	4.8
	20 - 39	8	9.5
	40 - 59	31	36.9
	≥ 60	41	48.8
Median age		56.2 ± 16.4	
Gender	Male	61	72.6
	Female	23	27.4

85.7% of the patients in the study were ≥ 40 years old, with a median age of 56.2 ± 16.4. Male patients accounted for 72.6%, and females made up 27.4%; the male/female ratio was 2.65.

** Endoscopic imaging characteristics of larger 10 mm colorectal polyps:*

We only chose the biggest polyps from each of the 84 individuals who had colorectal polyps to report endoscopic imaging and histology.

Table 2: Endoscopic imaging characteristics of colorectal polyps.

Polyp location	**Number of polyps (n = 84)**	**Percentage (%)**
Proximal colon	9	10.7
Distal colon	75	89.3
Polyp shape	**Number of polyps (n = 84)**	**Percentage (%)**
Sessile	4	4.8
Semi- pedunculated	11	13.1
Pedunculated	69	82.1
Polyp size	**Number of polyps (n = 84)**	**Percentage (%)**
10 - 20 mm	67	79.8
larger 20 mm	17	20.2
Medium size (mm)	18.3 ± 6.1	

Distal colon polyps larger than 10 mm were seen in 75 patients (89.3%), of which mainly in the sigmoid colon (50%) and rectum (32.1%). In terms of shape, 82.1% was pedunculate, while the proportion of semi-pedunculate and sessile polyps was 13.1% and 4.8%, respectively. Polyps 10 - 20 mm accounted for the largest proportion (79.8%).

** Histopathological characteristics of larger 10 mm colorectal polyps:*

Table 3: Characteristics of histopathology of above 10 mm colorectal polyps.

Histopathology		**Number of polyps**	**Percentage (%)**
Adenomatous polyps (n = 71)	Tubular adenoma	65	91.6
	Tubulovillous adenoma	5	7.0
	Villous adenoma	1	1.4
Non-adenomatous polyps (n = 13)	Polyps with hyperplasia	4	30.8
	Juvenile polyps	7	53.8
	Peutz - Jeghers polyp	2	15.4
Grade of dysplasia	Low	58	81.7
	High	13	18.3

Adenomatous polyps accounted for 84.5% mainly, of which tubular adenoma accounted for the highest proportion with 91.6%, polyps with a villous component were the lowest (8.4%) with 81.7% of low-grade dysplasia and 18.3% of high-grade dysplasia.

Juvenile polyps accounted for the largest proportion of non-adenomatous polyps (58.3%).

2. Prevalence of *KRAS* mutation and the association with endoscopic and histopathologic imaging

Table 4: Prevalence of KRAS gene mutations in patients with colorectal polyps larger than 10 mm.

Mutation status	Number (n)	Percentage (%)
Mutation	9	10.7
Non-mutation	75	89.3
Total	84	100

The prevalence of *KRAS* mutations in patients with colorectal polyps larger than 10mm was 10.7%.

Table 5: Association of KRAS gene mutations with endoscopic and histopathology features colorectal polyps larger than 10 mm.

Features of endoscopy and histopathology		*KRAS* mutation status		p
		No mutations (n, %)	Mutations (n, %)	
Polyp location (n = 84)	Proximal colon	9 (100)	0 (0)	> 0.05
	Distal colon	66 (88.0)	9 (12.0)	
Polyp shape (n = 84)	Pedunculated	61 (88.4)	8 (11.6)	
	Semi-pedunculated and sessile	14 (99.3)	1 (6.7)	
Polyp size (n = 84)	10 - 20 mm	60 (89.6)	7 (10.4)	
	> 20 mm	15 (88.2)	2 (11.8)	
Histopathology (n = 84)	Non-adenomatous	63 (88.7)	8 (11.3)	
	Adenomatous	12 (92.3)	1 (7.7)	
Histopathology of adenoma (n = 71)	Tubular adenoma	59 (90.8)	6 (9.2)	
	Adenoma with villous component	4 (66.7)	2 (33.3)	
Dysplasia (n = 71)	Low	53 (91.4)	5 (8.6)	
	High	10 (76.9)	3 (23.1)	

There were no associations of *KRAS* mutations with polyp features on endoscopy and histopathology (p > 0.05).

DISCUSSION

1. Characteristics of age, gender, colonoscopic images, and histopathology

** Age, gender:*

The research included 84 patients with an average age of 56.2 ± 16.4, a rate of 48.8% for those over 60, and a rate of 4.8% for those under 20. Therefore, the frequency of colorectal polyps increases with age. Our findings concur with those of other domestic and international authors [6, 7, 8, 9].

The ratio of male/female patients is 2.65/1, men accounted for 72.6%, and females accounted for 27.4%. Many national and foreign studies have also recorded a greater incidence of colorectal polyps in men than in women [7, 8, 9].

** Characteristics of larger 10mm polyps on endoscopy:*

In 84 patients with polyps larger than 10 mm on colonoscopy, the most common site was the sigmoid colon (50.0%), then the rectum (32.1%), and polyp in the proximal colon was less common. There are 79.8% polyps with a diameter of 10 - 20 mm, with the rate of the pedunculated polyp being 82.1%. Our research results are consistent with domestic studies such as the study of Vo Hong Minh Cong (2015), showing that polyps larger than 10 mm are also mainly seen in 2 locations, the sigmoid colon (34.7%) and rectum (31.9%), with the main size from 10 - 15 mm (accounting for 58.3%), the percentage of polyps larger 20 mm was 22.3% [6].

** Histopathological characteristics of colorectal polyps larger than 10 mm:*

According to the study's histopathological imaging, tubular-adenoma accounted for the largest percentage (91.6%) of all adenomatous polyps, whereas the rates of tubulovillous and villous adenoma were lower (7.0% and 1.4%, respectively). According to the majority of research, tubular adenomas predominate and are the most prevalent kind of adenomatous polyps. The occurrence of villous adenoma is typically relatively low. However, this kind should be observed due to the danger of malignancy transformation [3].

According to the WHO classification of dysplasia for individuals with adenoma in 2010, high-grade dysplasia accounted for 18.3% of all cases. High-grade dysplasia is regarded as precancerous, but the incidence is less frequent than in Vo Hong Minh Cong's (2015) study on a group of polyps larger than 10 mm, where the rate of severe dysplasia was 21.8% [6] and study of Vu Van Khien et al (2016), where 14% of polyps larger than 2 cm had severe dysplasia, while moderate and mild dysplasia accounted for 50.4% and 35.6%, respectively [7]. Foreign research also demonstrated the

low prevalence of high-grade dysplasia adenoma, a study by Basnet D. et al. (2021) on 61 adenoma, high-grate dysplasia was only 6.6% [8], a study by Tamannna K. et al. (2016) on 88 adenomas, this type was 10.2% [9]. Accordingly, large-sized polyp histopathology is crucial for identifying dysplasia and determining the best course of therapy, as well as for monitoring and screening [3].

2. *KRAS* mutation rate in patients with colorectal polyps over 10 mm in size

More than 3000 *KRAS* point mutations have been documented, with codon 12 and codon 13 in exon 2 being the most often affected. Codon 12 and 13 mutations are critical for cancer development and increase the chance of EGFR inhibitor drug resistance [2].

Using RNA samples, we found *KRAS* mutations in codons 12 and 13 in 84 samples of colorectal polyps larger than 10 mm in 84 individuals. The study's findings revealed that individuals with colorectal polyps had a 10.7% mutation rate in the *KRAS* gene and 100% mutations in codon 12. Between investigations, there were variations in the detection of *KRAS* mutations in patients with colorectal polyps.

Table 6: Comparison of KRAS mutation rates in patients with colorectal polyps of some authors.

Author (year)	Mutation identification techniques	Mutation rate (%)
Maltzman, T. et al. (2001) [10]	Gene sequencing	17.2
Barry E.L.R. et al. (2006) [11]	dHPLC + Gene Sequencing	3.0
Lorentzen J.A. et al. (2016) [12]	Sanger Sequencing	26.2
Our (2022)	RNA sample analysis	10.7

The frequency of *KRAS* mutations in patients with colorectal polyps, therefore, varies between studies' findings. The mutation depends on the characteristics of the study sample, which has a higher proportion of large-sized polyps and a different villous component, the method of identifying gene mutation, as well as the race and habit... of the study's subject [10, 11, 12].

3. Association of *KRAS* mutations with endoscopic imaging and histopathology of over 10mm colorectal polyps

We have not recognized an association between *KRAS* mutations and the location, shape, and size of polyps on endoscopy. The size of the polyp has a direct correlation with the risk of *KRAS* mutations and the possibility of cancer development, according to studies conducted throughout the world. The location and form of polyps do not impact the likelihood of *KRAS* mutations. Lorentzen J.A. et al. (2016) examined 172 adenomas and discovered that the rate of *KRAS* mutations in colorectal polyps larger than 10 mm was 32.6%, as opposed to 18.2% in the group of polyps 5 to 9 mm, with a p-value of 0.03. According to a subgroup study of 140 tubular adenomas, the rate of *KRAS* mutations was 9.0% in the group of polyps from 5 to 9 mm and increased to 24.7% in the group of polyps measuring 10 mm or more, with a p-value of 0.014 [12]. Maltzman T. et al. (2001) observed in a study of 738 adenomas that the *KRAS* mutation rate increased gradually with polyp size from 11.0% in polyps 1cm to 19.7% in polyps 1 - 1.49 cm, 24.4% in polyps 1.5 - 1.99 cm, and as high as 29.1% in polyps 2 cm, $p < 0.001$. Compared to polyps under 1 cm, polyps larger than 2 cm were 3.3 times more likely to have *KRAS* mutations (95%CI = 1.8 - 6.1). However, the size of adenomas did not independently correlate with *KRAS* mutations when multivariate analysis was adjusted for histopathological features ($p = 0.17$) [10].

Regarding the association of *KRAS* mutations with histopathology of polyps, studies have shown that polyps with a villous component and high-grade dysplasia carry more *KRAS* mutations, such as the study by Maltzman T. et al. (2001) noted that *KRAS* mutations appeared in 27.7% of adenoma with a villous component, higher than the corresponding rate in the group of tubular adenoma of 10.6% (OR = 3.2, 95%CI = 2.1 - 4.9, $p < 0.05$) and the mutation rate of high-grade dysplastic polyps was 32.0%, also higher than in the group of low-grade dysplastic polyps with 13.6% (OR = 3.0, 95%CI = 1.9 - 4.6, $p < 0.05$) [10]. The results of our study also noted higher rates of *KRAS* mutations in polyps with a villous component compared to tubular adenoma (33.3% vs. 9.2%) and high-grade dysplastic polyps compared with

low-grade dysplastic polyps (23.1% vs. 8.6%). As a result, mutations in the *KRAS* gene may be an important factor in the progression of adenoma to the villous component and high-grade dysplasia, potential factors for colorectal cancer [2].

CONCLUSION

We get the following results after examining 84 samples of DNA polyps larger than 10 mm for *KRAS* mutations:

- The frequency of *KRAS* mutations is 10.7%.

- There is no evidence linking *KRAS* mutations to specific polyp features seen in endoscopic imaging.

- The prevalence of *KRAS* mutations was higher in polyps with a villous component compared to tubular adenoma (33.3% vs. 9.2%) and high-grade dysplastic polyps with low-grade dysplastic polyps (23.1% vs. 8.6%).

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