KRAS mutations and their associations with clinicopathological features and survival in Vietnamese non-polyp colon cancer patients

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Abstract. The aim of the present study was to determine Kirsten Ras sarcoma virus (*KRAS*) mutations and the associations of *KRAS* mutations with clinicopathological features and treatment outcomes in Vietnamese non-polyp colon cancer (NPCC) patients. The data in the present study covered 194 patients with non-polyp colon cancers at stages II or III, according to the 8th edition of the American Joint Committee on Cancer staging system, in northern Vietnam from January 2016 to August 2020. All patients underwent radical surgery and adjuvant therapy with FOLFOX4 or XELOX. Subsequently, the recruited patients were followed-up with scheduled hospital exams for diagnosing recurrence. Genomic DNA samples were prepared from dissected tumors and specific sequences of the *KRAS* gene were amplified by polymerase chain reactions (PCR). The mutations at codons 12, 13, 59, 60, 61, 117 and 146 of the gene were determined. Possible associations of the *KRAS* mutations with clinicopathological properties and the survival of patients were analysed. The *KRAS* mutation rate was 47.9% in Vietnamese patients with NPCC, of those, mutations in exon 2 accounted for 91.4% of all detected mutations. The mutated-KRAS patients exhibited a significantly higher rate of anemia. Moreover, the *KRAS* mutation rate was higher in females (57.1%) than in males (39.8%). The *KRAS* mutation rate was also higher in patients with right colon cancers. Furthermore, *KRAS* mutations were an independent prognosis for poor disease-free survival (DFS) and overall survival (OS) in stage II patients. Among left-sided colon patients, mutated *KRAS* was a significant predictive factor for poor DFS but not for OS. The present study revealed a very high mutation rate of *KRAS* in Vietnamese patients with NPCC. The data of the present study indicated that the mutation status was associated with female patients and right-sided tumors. The *KRAS* mutations were a negative factor for the survival of patients with stage II NPCC and patients with left-sided colon cancer.

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Introduction

Colon cancer (CC) is among the five most common cancers in Vietnam and globally. In 2020, 1,148,515 new cases were reported and 576,858 deaths were attributed to CC worldwide (1). CC is the fourth deadliest cancer in Vietnam (both sexes combined), with an increasing tendency of new cases (2,3). Biomarker discoveries have provided the possibility for early diagnosis and partly supported mortality reduction of CC (4). Among important discovered oncogenes, *KRAS* proto-oncogene, a homolog of Kirsten Ras sarcoma virus (*KRAS*) oncogene in humans, has been showing the highest detected mutation rate in CC with >35% of patients (5-7). Most of the mutations at these sites continuously trigger mitogen-activated protein kinase (MAPK) pathways and subsequently, the proliferation of cancer cells (8-12). The presence of a KRAS mutation is a predictive factor for resistance to anti-epidermal growth factor receptor (EGFR) treatment. Therefore, the mutated gene is considered a worse prognosis in advanced colon cancer (13-17).

However, the impact of KRAS mutation is controversial in colon cancer stage I-III. The worse prognosis for survival has been indicated in some research, particularly in some subgroups determined by disease stage, tumor site, sex, BRAF wild-type, and microsatellite instability (MSI) status (18-21), while other studies have not shown these associations (22-24).

KRAS status has been described in colonic polyps and KRAS mutations were listed as a potential molecular factor for the risk of developing advanced neoplasia (25,26). Currently, the progression from polyp to cancer has been

identified. The development of normal epithelial cells to adenocarcinoma generally follows a progression of histological and concurrent epigenetic and genetic changes (27). Colon cancer without the occurrence of polyps has different pathogeneses compared with polyposis syndrome colon cancer (28,29). Non-polyp status was examined by colonoscopy and/or confirmed by observing the dissected tissues after surgery. By gathering the results of this group, patients with certain polyposis syndrome were excluded from the present research.

Due to the promotion of KRAS gene mutations to the tumor invasion and metastatic processes (30), numerous studies have referred to the KRAS gene as a worse prognostic factor in survival, worldwide. However, few studies on *KRAS* mutations in Vietnamese patients with CC have been reported and the *KRAS* status for colon cancer without the occurrence of polyps has not been described (31,32). In addition, the associations of the *KRAS* status with the survival of the Vietnamese patients with NPCC are also under establishment. Therefore, the present study aimed to determine *KRAS* status and possible associations of KRAS status with clinicopathological features and survival in non-polyp colon cancer stage II-III in patients from Vietnam.

Materials and methods

Data in the present study were collected from 194 patients (males, 53.1% and females, 46.9%; median age, 58 years) with non-polyp colon cancers at stages II or III from January 2016 to August 2020 at The Nuclear Medicine and Oncology Center of Bach Mai Hospital, The Oncology Department of Viet Duc Hospital, and National Cancer Hospital (Hanoi, Vietnam). The inclusion criteria were as follows: i) Stages II-III colon cancer according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system; ii) no occurrence of polyps; iii) radical surgery and adjuvant therapy with FOLFOX-4 or XELOX; iv) testing for the *KRAS* status; and v) access to the medical records of patients. The exclusion criteria were as follows: i) Diagnosed with a second cancer; ii) medical history indicating the removal of colorectal polyps; iii) patients with the appearance of colon polyps in any numbers at any time; and iv) inability to answer the research questions due to illness. The present study was approved (approval no. NCS28/HMU-IRB) by the Ethics Committee of Hanoi Medical University (Hanoi, Vietnam). Written informed consent was obtained from all participants.

All patients underwent radical treatment and followed-up with a scheduled exam at hospitals. Studied variables included clinical and subclinical features such as age, sex, tumor site (33), histopathology (34), cancer stage, and *KRAS* status.

DNA isolation and mutation analysis. KRAS mutations were investigated with 10% formalin-fixed (carried out at room temperature for ~12 h), paraffin-embedded (FFPE) tissue. A total of five of 10-μm-thick tissue slides from each FFPE tissue block (194 samples) were used for the isolation of genomic DNA. Paraffin in FFPE tissue slides was removed using an FFPE deparaffinization solution (Merck KGaA). The DNA was then extracted from tissue samples with the aid of PureLink™ Genomic DNA Mini Kit according to the manufacturer's instructions (Invitrogen; Thermo Fisher Scientific, Inc.).

KRAS mutations were detected with KRAS XL StripAssay (ViennaLab Diagnostics GmbH) in a five-step procedure including amplification, hybridization, stringent wash and color development. Briefly, prepared DNA samples were mixed with Taq-polymerase (ViennaLab Diagnostics GmbH) and an amplification mixture from the manufacturer. The specific DNA sequences were amplified in 35 cycles of polymerase chain reactions (PCR). Pre-PCR was performed at 37˚C for 10 min and 94˚C for 2 min. The thermocycling conditions were as follows: 94˚C for 1 min, 70˚C for 50 sec, 56˚C for 50 sec, 60˚C for 1 min (35 cycles) and a final extension at 60˚C for 3 min. The amplification products were stored on ice or at 2-8˚C until further use. The PCR products were then denatured and hybridized with probes on strips in the hybridization buffer. After hybridization ended, solutions were removed, and the strips were washed. The conjugated solution was then added to the strips, followed by a second washing step. Lastly, the color developer was added to the strips in the dark for the appearance of purple positive bands, followed by a third washing step. The strips were analyzed using the KRAS collector sheet included in the kit box or StripAssay Evaluator software (version 2.12.2018.212) to determine KRAS mutation at codons 12, 13, 59, 60, 61, 117, and 146. Both the collector sheet and the software were developed and provided by ViennaLab Diagnostics GmbH, Austria (35,36).

Statistical analysis. Statistical analysis was performed using SPSS 21.0 (IBM Corp.). Non-normal variables (age) were reported as a median. Nonparametric tests were used to compare the median of two groups of non-normal distribution. Differences between groups were assessed using the Chi-square tests. All tests were two-sided, with a significance level of $P<0.05$. Multivariate analysis was estimated using binary logistic regression models. The Kaplan-Meier method was used to calculate the survival rate, and the log-rank test was performed to compare survival rates. Cox's regression model with a 95% confidence interval (CI) was used for multivariate survival analysis. A $P<0.05$ was considered to indicate a statistically significant difference.

Results

During the course of the study, a total of 194 patients who satisfied the selected conditions were included in the analysis. Among them, 91 (46.9%) were females, of whom 39 (20.1%) carried the wild-type while 52 (26.8%) had the mutated *KRAS*. On the other hand, 103 (53.1%) were males including 62 (32.0%) wild-type and 41 (21.1%) mutated *KRAS*. The age of the patients ranged from 24 to 75 with a median age of 58-years-old. As summarized in Fig. 1, the minimum age in the mutated *KRAS*-carrying males and females was lower than those of the patients with the wild-type *KRAS*. The median age of the mutated *KRAS* patients was 57 (58 for males; 56 for females) which was also lower than the 59 for the wild-type *KRAS* group (60 for males; 57 for females). Furthermore, pair-wise comparisons using nonparametric tests showed no

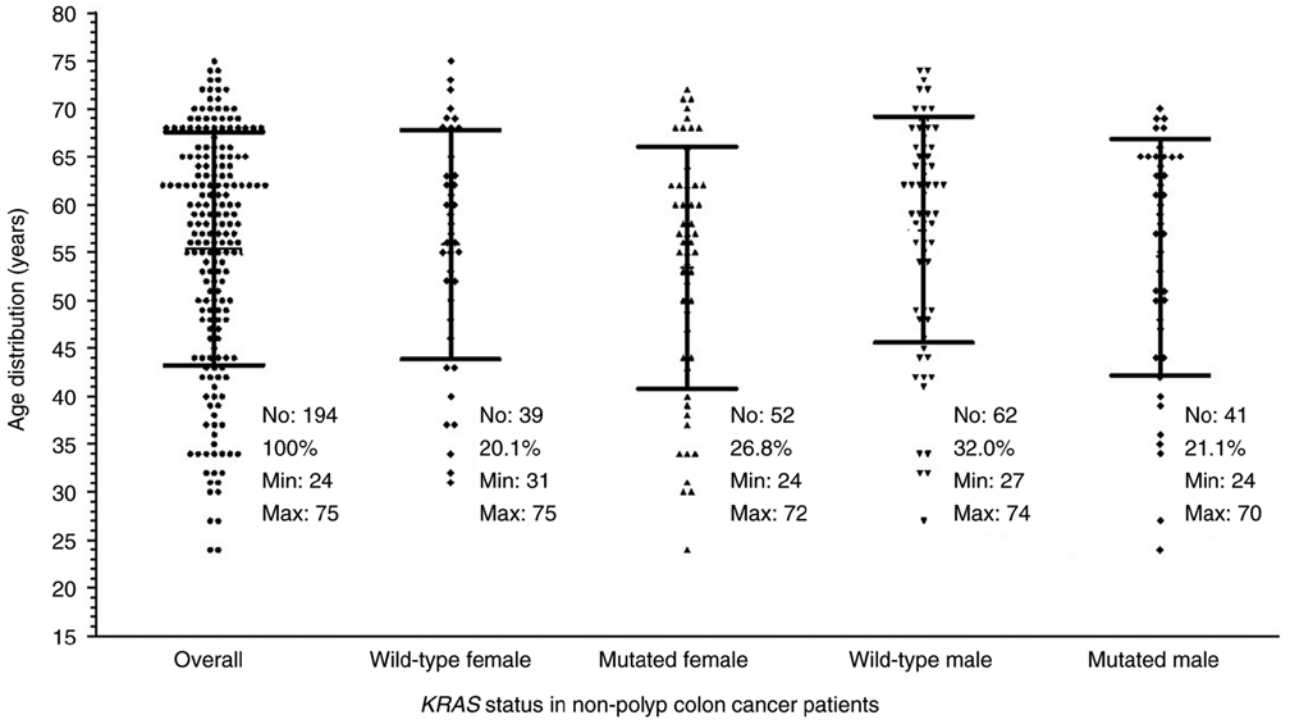

Figure 1. Age distributions by sex per the *KRAS* status of patients with non-polyp colon cancer. The bars indicated the means of ages with standard deviations. Wild-type groups represented the groups with wild-type *KRAS* and the mutated groups indicated that one of the *KRAS* mutations was detected.

significant differences between the mutated and the wild-type groups, even in subgroups by sex.

Mutations of the KRAS gene in Vietnamese patients with NPCC at stages II and III. Point mutations of the *KRAS* gene in exon 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) were determined. Overall, the *KRAS* mutation was identified in 47.9% of the patients (Fig. 2). Of which, mutations in exon 2 were dominant with 85 cases accounting for 43.8% of recruited patients (or 91.4% of the total detected mutations). Mutations in exon 3 including 1 case at codon 59 and 5 cases at codon 61 resulted in 3.1%. Only two cases of mutated exon 4 were detected (1%), one carried mutation at codon 117 and the other at codon 146 (data not shown). None of the patients had more than one mutation.

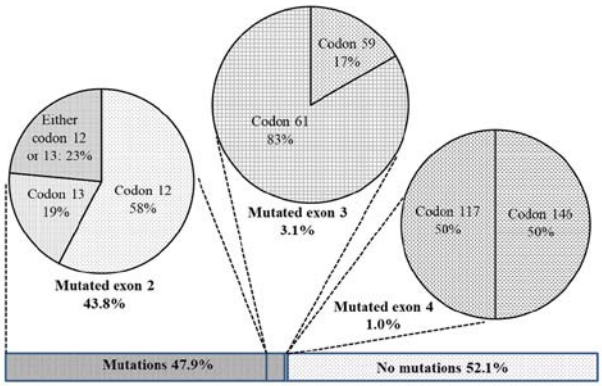

Figure 2. *KRAS* mutations in Vietnamese patients with non-polyp colon cancer.

Associations of KRAS status and clinical features. A total of seven clinical symptoms were recorded before surgery and displayed in Fig. 3. Abdominal pain was predominant among the symptoms with 157 cases, accounting for 80.9% of patients. Diarrhea was the second most common symptom that was reported in 51 cases (26.3%). Anemia and weight loss were lower with 42 (21.6%) and 39 (20.1%) cases, respectively. Other notable symptoms were bleeding with 36 cases (18.6%), blood in stool with 33 cases (17%) and constipation with 36 cases (18.6%).

A pair-wise comparison between the wild-type and mutated *KRAS* groups for each symptom was conducted to identify the possible association of a symptom with the *KRAS* status. Most of the observed symptoms were unlikely to be correlated with the *KRAS* mutations (Fig. 3). Abdominal pains were highly recorded in both groups with 84.2 and 77.4% in the wild-type group and mutation groups, respectively. Other symptoms which did not exhibit considerable changes between the two groups included bleeding (14.9 vs. 22.6%), blood in the stool (21.8 vs. 11.8%), diarrhea (25.7 vs. 26.9%), constipation (19.8 vs. 17.2%) and weight loss (19.8 vs. 20.4%), respectively. However, the analysis revealed a significant increase in cases of anemia among the mutated group with P=0.041. Particularly, 28.0% of mutated *KRAS* patients exhibited anemia symptoms while only 15.8% of the wild-type group did (Fig. 3), indicating an association of anemia with *KRAS* mutation in NPCC.

Association of KRAS mutation with clinicopathological features in patients with NPCC. The possible associations of clinicopathological features with *KRAS* status in patients with NPCC is revealed in Table I. Notably, the *KRAS* mutation rate in females was statistically higher than in males. With

Table I. Clinicopathological features and *KRAS* status.

Characteristic	Total (n=194)	*KRAS* mutation status: Wild type (n=101, 52.1%)	*KRAS* mutation status: Mutated (n=93, 47.9%)	P-value
Age				0.714
<50	56	28 (27.7%)	28 (30.1%)	
≥50	138	73 (72.3%)	65 (69.9%)	
Sex				0.016[a]
Male	103	62 (61.4%)	41 (44.1%)	
Female	91	39 (38.6%)	52 (55.9%)	
Tumour sites				0.047[a]
Right	92	41 (40.6%)	51 (54.8%)	
Left	102	60 (59.4%)	42 (45.2%)	
Histological types				0.583
Adenocarcinoma	164	84 (83.2%)	80 (86.0%)	
Others	30	17 (16.8%)	13 (14.0%)	
Invasion depth				0.333
T3	81	47 (46.5%)	34 (36.6%)	
T4a	92	43 (42.6%)	49 (52.7%)	
T4b	21	11 (10.9%)	10 (10.8%)	
Lymphnode status				0.190
N0	93	52 (51.5%)	41 (44.1%)	
N1	71	32 (30.7%)	40 (43.0%)	
N2	30	18 (17.8%)	12 (12.9%)	
pTNM stage				0.303
II	93	52 (51.5%)	41 (44.1%)	
III	101	49 (48.5%)	52 (55.9%)	

[a]P<0.05.

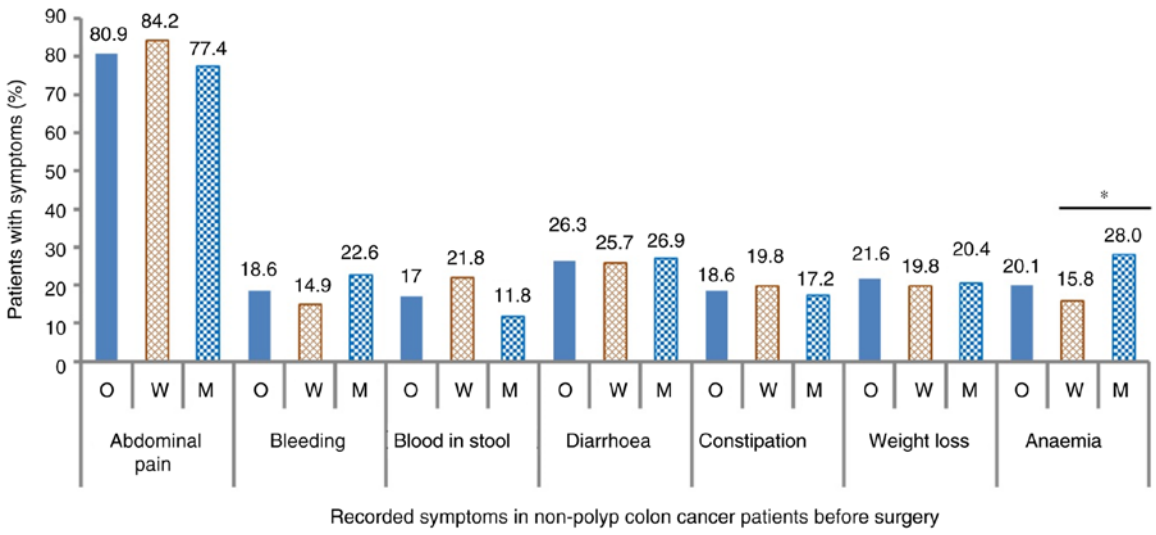

Figure 3. Clinical properties of patients with non-polyp colon cancer before surgery based on *KRAS* status. O indicates overall (total) data, W represents the wild-type, whereas M represents the mutated groups. *P<0.05.

52 females, the *KRAS* mutation rate was identified in 55.9% of the cases, far higher than 44.1% identified in the males. The mutations were also detected at a higher rate in right-sided cancers (RC) compared with left-sided cancers (LC) (54.8%

Table II. Associations between *KRAS* mutation and prognostic markers.

KRAS mutation		Multivariate logistic regression		
		OR	95% CI	P-value
Age	≥50/<50	0.821	0.426-1.582	0.555
Sex	Female/Male	2.144	1.184-3.882	0.012[a]
Tumour location	Left/Right	0.551	0.305-0.996	0.048[a]
Invasion depth	T4/T3	1.421	0.776-2.600	0.255
Nodal status	N (+)/(-)	1.239	0.681-2.253	0.483
Histology	Adenocarcinoma/Others	1.726	0.740-4.026	0.206

[a]P<0.05. OR, odds ratio; CI, confidence interval.

for the RC vs. 45.2% for the LC; P=0.047). By contrast, *KRAS* mutation rates were relatively compatible among the younger and older patients.

A high rate of adenocarcinoma (AC) was revealed in 164 cases (84.5% of patients) while other types were identified in 30 cases (15.5% of patients) (Table I). The number of NPCC AC cases in mutated vs. wild-type *KRAS* was almost identical, 80 and 84 cases, respectively, indicating that the *KRAS* mutation unlikely caused AC. The *KRAS* mutation rate among the AC patients with NPCC was also not different from that of the other types.

The invasion depth of tumors in the patients with NPCC could possibly be linked to the *KRAS* mutation. The T4 stage (including T4a and b) was confirmed in 59 cases of the mutated group, accounting for 63.5%. This stage was confirmed in only 54 wild-type patients (53.5% of the wild-type group). Similarly, stage III tumors were determined with a higher rate among the mutated patients (52 out of 93 cases, or 55.9%) compared with the same stage among the wild-type patients (49 out of 101 cases, or 48.5%). However, none of the associations between the staging and *KRAS* mutation were statistically significant, including lymph node status (Table I).

Multivariate regression analysis: Correlations of KRAS mutations with other prognostic markers. The association of *KRAS* mutations in females among Vietnamese patients with NPCC was confirmed by multivariate logistic regression. The *KRAS* mutation rate was significantly higher in females with an odds ratio (OR)=2.144 (95% CI: 1.184-3.882; P=0.012]. The analysis further confirmed the correlation of *KRAS* mutations with the right NPCC with P=0.048. Therein, the OR of left-sided colon cancer (LCC) to right-sided colon cancer (RCC) was 0.551 (95% CI: 0.305-0.996). In addition to the differences aforementioned, there was no evidence of an association between tumor staging and histologic types with *KRAS* mutation among Vietnamese patients with NPCC (Table II).

Treatment outcomes. During this study, one patient discontinued the follow-up examinations at the three aforementioned hospitals. Therefore, the treatment outcomes of 193 patients including 100 (51.8%) wild-type and 93 (48.2%) mutated *KRAS* cases were analyzed. The mean follow-up duration was 38.8±13.2 months (min, 12 months; max, 78 months). The averages of disease-free survival (DFS) and overall survival (OS) were 48.9±2.3 and 56.1±2.2 months, respectively. For patients with a 3-year follow-up, the DFS was 53.8% and the OS was 73.0% (data not shown).

Association between KRAS status and survival. Sorting patients into two groups by only their *KRAS* status revealed that mutated *KRAS* resulted in a worse trend for survival than the wild-type. The 3-year DFS of the mutated *KRAS* group was 48.8% while for that of the wild-type group it was 58.3%. However, the difference was not statistically significant (P=0.205). Similarly, the 4-year OS of the mutated *KRAS* group and wild-type groups were 63.0 and 67.0%, respectively (P=0.525) (data not shown).

Different and notable statistical data was observed after sorting patients into smaller groups with different criteria. As could be observed from Table III, *KRAS* mutation was associated with lower ratios of 3-year DFS and 4-year OS in patients with NPCC stage II. Among stage II patients, 53.2% of the mutated *KRAS* group had 3-year DFS, notably lower than that of the wild-type group (79.8%) with P=0.008. Similarly, the 4-year OS ratios of mutated *KRAS* and wild-type groups were 66.0 and 94.1%, respectively (P=0.021).

Significant changes were also observed in groups of patients with LCC. The 3-year DFS) of the mutated *KRAS* LCC group was 32.2% compared with 55.9% of the wild-type *KRAS* LCC group (P=0.012). The 4-year OS rates of the mutated and wild-type *KRAS* LCC were 52.8 and 72.7%, respectively (P=0.070). In addition, the effect of *KRAS* mutations on the survival of patients did not significantly differ according to age groups, sexes, RCC, histopathology of AC, and tumor stages (Table III).

Further analyses were performed for stage II patients using the Cox's model. The *KRAS* mutations were independently related to worse DFS and OS. The hazard rate (HR) of DFS was 3.353 (95% CI: 1.480-7.594; P=0.004; Fig. 4A), and that of OS was 3.640 (95% CI: 1.130-11.722, P=0.030; Fig. 4B).

Discussion

The association of mutations in the *KRAS* gene with poor DFS of colon cancer has been well-studied worldwide (37). More

Table III. Associations between *KRAS* status and survival in specified groups.

		3-year DFS			4-year OS		
Specified groups (n)	n	*KRAS* wt (%)	Mutated *KRAS* (%)	P-value	*KRAS* wt (%)	Mutated *KRAS* (%)	P-value
Age <50	56	60.3	46.2	0.364	67.6	63.7	0.254
Age ≥50	137	57.7	50.0	0.376	63.7	63.4	0.937
Male	102	55.0	51.2	0.658	66.9	61.2	0.455
Female	91	64.1	46.6	0.146	67.3	65.7	0.798
Right-sided	91	62.3	62.4	0.795	56.6	73.9	0.429
Left-sided	102	55.9	32.2	0.012[a]	72.7	52.8	0.070[a]
Adenocarcinoma	163	63.2	51.0	0.124	72.5	66.6	0.318
pT3	81	71.4	73.4	0.946	86.8	85.3	0.671
pT4	112	46.7	34.7	0.301	50.0	52.4	0.877
Stage II	92	79.8	53.2	0.008[a]	94.1	66.0	0.021[a]
Stage III	101	35.7	45.3	0.379	36.8	60.3	0.184

[a]P<0.05. P-value, log-rank testing for DFS. OS, overall survival; DFS, disease-free survival.

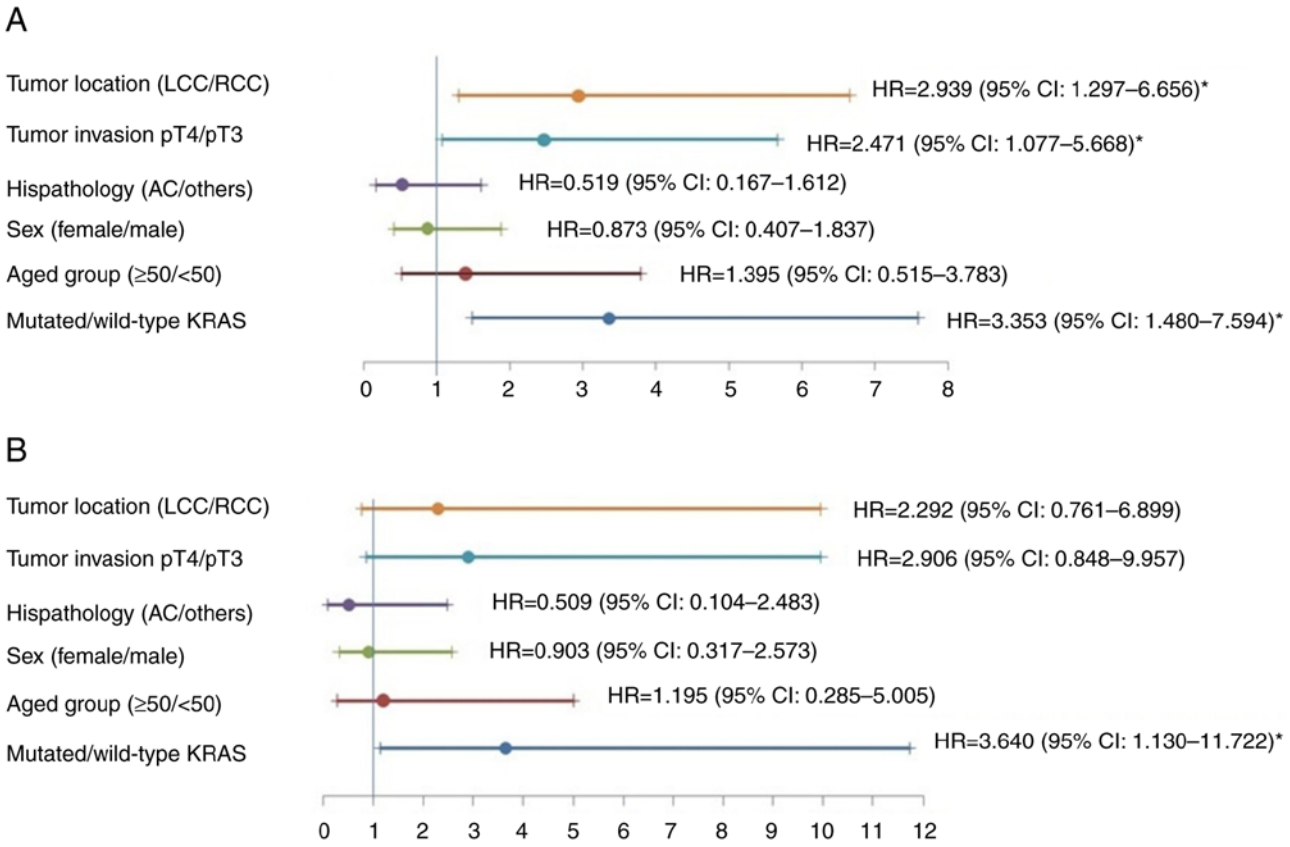

Figure 4. Cox regression analysis for (A) disease-free survival and (B) overall survival in patients with non-polyp colon cancer stage II. *P<0.05.

than 3000-point mutations of the *KRAS* gene in colorectal cancer are reported in the literature. Most (~82%) reported mutations were in codon 12. Mutations at codons 13 and 61 accounted for ~17 and ~1%, respectively (18,38,39). A meta-analysis, by Roth *et al*, of four large clinical trials in CC stage II-III patients who underwent radical treatment indicated the *KRAS* mutation rate was 37.0% (36.0% in stage II, 37.5% in stage III) (22). Another study on 228 patients with CC showed that the rate of mutated *KRAS* was 39.9%, of which 26.0% was in codon 12, 6.6% in codon 13, and 3.5% in codon 61 (21). The rate of mutated *KRAS* in Vietnamese patients with CC was 41.0, and >80% of mutations were detected at codon 12 or 13 in Exon 2 (56/64), while a minority (8 cases) was identified at codon 61 in Exon 3 (31).

However, the *KRAS* mutation rate in NPCC has rarely been reported in Vietnam. According to the results of the present

study in which point mutations of *KRAS* in exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) were determined, the overall *KRAS* mutation rate was identified to be 47.9% in patients with NPCC (Fig. 2), notably a high rate in the country. This detected rate was surprisingly higher than previous studies reporting 36-38% *KRAS* mutation rates (5,21,22). The mutations mostly occurred in exon 2 of the gene. Particularly, the mutation rate in *KRAS* exon 2 was 43.8% of the recruited patients, markedly higher than a previous study on CC in Vietnam (37.1%) and China (42.2%) (32,40). The mutation rate in *KRAS* exon 3 (3.1%) was relatively similar to that reported in a study by Guo *et al* (40). While the mutations in exon 4 were rare in Vietnamese patients with NPCC with only 2 cases, or 1% reported in the present study.

Clinicopathological properties such as abdominal pain, anemia and various symptoms were identified in >83% of patients. Of those, diarrhea and anemia were likely correlated to the tumors in the right colon (41). Logically, an association between diarrhea and anemia with *KRAS* mutations is suspected. However, the current data has confirmed significantly higher anemia cases but not diarrhea cases among *KRAS*-mutated patients with NPCC. Mutated KRAS had a higher frequency in the right-side colon compared with the left-side colon.

Another impressive finding in the present study was the association of *KRAS* mutations with female patients. The mutations were detected in 57.1% of female patients (52 out of 91 females) and only 39.8% of male patients (41 out of 103 males). Females accounted for 55.9% of patients with mutated *KRAS* genes (Table I) while males accounted for 44.1%. These sex ratios among patients with mutated *KRAS* genes were contrary to those in a previous study for CC in Vietnam, which reported 48.2 and 51.8% for female and male patients with mutated *KRAS* genes, respectively (32). In addition, the age of *KRAS* mutation-carrying patients in each sex was lower than those of the wild-type groups. However, the correlation of *KRAS* mutation with the age of patients was unclear. By applying multivariate logistic regression, the *KRAS* mutation rate was significantly higher in females, and the analysis further confirmed the prevalence of *KRAS* mutations in the right-side colon (Table II).

Several studies have revealed that the *KRAS* mutation rate was likely higher in the RCC than in the LCC (5,20,42). In the present study, the association of the *KRAS* mutation rate with the RCC was clarified. The *KRAS* mutation rate in the RCC was 55.4% (51 mutants out of 92), while the rate in the LCC was only 41.2% (42 mutants out of 102).

The effect of *KRAS* mutation status on survival has been reported in previous studies; however, the results were controversial. Some authors indicated that *KRAS* mutations led to a worse prognosis of DFS in patients with CC at stages II-III (6,18,20,21). Conversely, an opposite conclusion concerning the prognostic value of the *KRAS* gene has been reported (22-24).

By analyzing some specified groups, the prognostic values of *KRAS* mutations in the distinct group of patients with NPCC stage II-III, were clarified. Most previous studies have reported that RCC was associated with a higher recurrence and lower survival rate than LCC. Some clinical features were considered to explain this issue, as in the earlier stage, fewer nodal invasions, and fewer gene mutations such as BRAF, PIK3CA, CTNNB1 and PTEN (33,43,44) were diagnosed. Among LCC patients, mutated *KRAS* was a significant predictive factor for poor DFS (32.2% of mutated *KRAS* vs. 55.9% of wild-type; P=0.012) but not for OS (Table III). Some other studies supported this result. Xie *et al* indicated mutated-KRAS was a worse prognosis for OS in LCC (HR: 1.21; 95% CI: 1.08-1.36; P<0.01) (42). In LCC stage IV, mutated KRAS was significantly related to a higher risk of mortality (HR: 1.18; 95% CI: 1.05-1.33) (45).

Although no considered difference in survival time was revealed in overall NPCC stage II-III patients according to *KRAS* mutation status, poor DFS and OS were observed in stage II patients. The Cox's regression analysis revealed that *KRAS* mutations were an independent prognostic factor for stage II patients (HR of DFS: 3.353; P=0.004; HR of OS: 3.640; P=0.030; Fig. 4). This data differed from that reported in Chinese patients with CC (40). A study by Natsume *et al* also reported survival in colon cancer stage II, with a 5-year DFS of mutated KRAS vs. KRAS wild-type: 75.9 vs. 76.7%; and a 5-year OS of 84.3 vs. 87.1% (P>0.05) (46). A study in early-stage colorectal cancer (stage I-II), with an average of 72 months of follow-up, revealed that DFS was worse in patients with KRAS codon 13 mutation (stage I: P=0.015; stage II: P<0.001) (47). The worse survival of KRAS-mutated colon cancer stage II could be explained by the role of the KRAS-mutated gene in colon cancer. KRAS gene mutations that occur in the earlier stage of colon cancer can cause earlier recurrence.

The limitations of the present study were the impact on survival, analyzed only by KRAS status, not in association with other gene mutations such as BRAF, NRAS and MMR, and with recurrent patients, the treatment after diagnosis was not standardized.

In conclusion, the *KRAS* mutation rate in NPCC stage II-III in Vietnam was very high (47.9%). Of which, mutations in exon 2 were dominant and detected in 43.8% of the recruited patients (or 91.4% of the total detected mutations). The patients who carried the mutated-*KRAS* further potentially experienced anemia. Moreover, the *KRAS* mutations occurred in Vietnamese females with NPCC and RCC patients. Furthermore, the mutated *KRAS* was an independent predictor for poor DFS and OS in stage II patients and DFS in LCC. These findings provide essential scientific background for treating and managing NPCC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HMC, VHT and NMD wrote the manuscript. HMC and VHT participated in design of the study. HMC, VHT and NTL were involved in acquisition of data. HMC, VHT and BTTH participated in analysis and interpretation of data. HMC, VHT, BTTH and NMD confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. NCS28/HMU-IRB) by the Ethics Committee of Hanoi Medical University (Hanoi, Vietnam). Written informed consent from all participants was obtained.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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