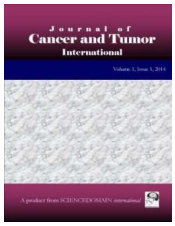

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Prognostic Value of *KRAS* Mutations in Codons 12 and 13 in South Egyptian Colorectal Cancer Patients

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to assess the frequency of Kirsten rat sarcoma viral oncogene homolog *(KRAS)* gene mutation, perform clinicopathologic characterization of *KRAS* mutated colorectal cancer (CRC), evaluation of the prognostic significance of *KRAS* mutations and their influences on the outcome in patients with CRC after curative surgical resection.
Patients and Methods: This study included 79 previously untreated CRC cases presented to South Egypt Cancer Institute from January 2011 to June 2013. Tumor samples were prospectively analyzed for *KRAS* mutations detection using DNA Clamp technique.
Results: Genotyping revealed an overall mutation frequency of 31.6% in *KRAS* gene. 68% of the mutations were in codon 12 and 23% in codon 13. Patients with *KRAS* mutated CRC had statistically significant increase in the rate of local recurrence (*P*=.002) and distant metastases (*P*=.024) compared to patients with *KRAS* wild type CRC. There was statistically significant poorer 2-year disease free survival (DFS) (61.6% vs 86.2%, *P*=.01) and overall survival (OS) (83.1% vs 90.7%, *P*=0.04) in cases with *KRAS* mutated vs *KRAS* wild type CRC. Codon 13 was associated

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with statistically significant lower 2-year DFS (46.9% vs 81.4%, *P*=.008) and OS (62.5% vs 91.4%, *P*=.009) compared to cases without codon 13 *KRAS* mutation.
Conclusion: The prognostic significance of *KRAS* mutation status regarding survival and treatment outcome in CRC might form the basis for more specific adjuvant therapy to improve survival.

Keywords: Colorectal cancer; KRAS mutations; prognostic factors; real time PCR.

1. INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer in men and the third most common in women [1]. Approximately 40% of CRCs harbor activating mutations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*), making it the most commonly mutated gene in the RAS/RAF/MAPK pathway. *KRAS* mutations are believed to be an early event in colorectal tumorigenesis and lead to constitutive signaling and downstream activation of MAPK- and PI3K-dependent pathways [2]. The *KRAS* encodes a 21-kDa protein (p21ras), which controls cell growth and differentiation by the transduction of extracellular mitogenic signals [3]. Mutations of *KRAS*, especially in codons 12, 13, and 61, have been detected in patients with CRC that lead to the formation of active (i.e. GTP-bound) proteins, which trigger the transduction of proliferative and/or differentiative signals even in absence of extracellular stimuli [4,5].

Ninety percent of *KRAS* mutations occur in codons 12 and 13 of exon 2, with the most frequent alteration being a G4A transition in codon 12 [6]. The role of *KRAS* as prognostic biomarker of CRC survival is controversial; some studies reported no association between *KRAS* mutation status and CRC survival [7,8], whereas others suggested poorer outcome for any mutation subtype [9].

Several studies have reported the predictive value of *KRAS* marker regarding the response to anti-epidermal growth factor receptor (*EGFR*) therapy in metastatic CRC cases [10,11]. However its role in localized disease is less clear; a phase III randomized trial of patients with stage III colon cancer demonstrated the prognostic value of *KRAS* mutation status {3-year disease free survival (DFS) was 72%-75% vs 65%-67% in *KRAS* wild and *KRAS* mutated respectively} irrespective of *anti-EGFR* therapy with no effect on the 3-year DFS [12].

The aim of the study was to assess the frequency of *KRAS* gene mutations, perform clinicopathologic characterization of *KRAS* mutated CRC, assess the prognostic significance of *KRAS* mutations and detect the influence of *KRAS* mutations on outcome in patients with CRC after curative surgical resection.

2. PATIENTS AND METHODS

The study population comprises 79 patients with completely resected CRC presented to South Egypt Cancer Institute from January 2011 to June 2013 and all patients were evaluated from January 2011 to January 2015.

2.1 Inclusion Criteria

- Previously untreated CRC
- Electively resected CRC (Tany, N1-2, M0)
- Histologically proven CRC
- Patients with a performance status ≤ 2 according to the Eastern Cooperative Oncology Group system.
- Adequate organ function (hemoglobin >11g/dl, total white blood cells >3,000/mm^3, absolute neutrophils >1,500/mm^3, and platelets >100,000/mm^3).

2.2 Exclusion Criteria

- Previous neoplasm
- Synchronous or metasynchronous metastasis (indicated as disease detected in the liver, lung, brain, and other organs outside the pelvis).
- Previous chemotherapy or radiotherapy before surgery

2.3 Diagnosis and Treatment

All patients underwent a complete clinical examination, including digital rectal examination, a chest X-ray, an abdomino-pelvic computed tomography (CT), barium enema, a transrectal ultrasound, proctosigmoidoscopy and colonoscopy. The diagnosis of CRC was histopathologically confirmed by endoscopic or laparotomy biopsies. Laboratory studies included: complete blood count, kidney function

tests, complete liver functions, random blood sugar, and coagulation profile.

Treatment modalities included surgery and adjuvant chemotherapy and radiotherapy.

2.3.1 Surgery

All patients were subjected to radical resection of the tumor with regional lymphadenectomy proximally up to vascular trunk. Surgical margins of at least 5 cm in colon cancer and 2 cm in rectal cancer were preserved. The tumor and the margins were examined histopathologically and subjected to *KRAS* mutation analysis. The pathologist assessed the tumor site, tumor size, stage, tumor grade, lymph node metastasis, and tumor type.

2.3.2 Postoperative Combined Chemo-radiation

2.3.2.1 Radiotherapy

Radiotherapy was delivered to all patients with rectal cancer after curative surgical resection.

2.3.2.1.1 Target volume

Included the tumor bed (rectum and the draining lymph node chains; pararectal, hypogastric, presacral lymph nodes) and was defined using the simulator. Target volume was localized with the patient in the prone position with a full bladder (to displace the small bowel anteriorly and superiorly). Patients underwent CT simulation in a prone position with distended bladder, belly board, and thermoplastic sheet fixation. Multiple CT cuts at 0.5 cm interval were obtained throughout the pelvis. CT data was transferred to the XiO treatment planning system (version 4.2). On each axial CT slice, clinical target volume (CTV) and organs at risk (OAR) were contoured. Planning target volume (PTV) was generated with a 0.5 cm expansion from the CTV to account for the setup errors. Appropriate field weighting and beam modifiers (wedges and blocks) were selected to keep the OAR doses below their tolerance.

2.3.2.1.2 Field arrangement

Three field techniques were used (one posterior and two opposing wedged lateral fields) to give a homogeneous distribution to the target volume and to spare the anterior structures, particularly the small bowel.

2.3.2.1.3 Dose and energy

All patients were treated by a photon beam of either 6 or 15 MeV generated from a linear accelerator (Siemens Mevatron). The dose was 45 Gray in 25 fractions over 5 weeks prescribed at the isocenter of the plan according to ICRU report No. 50.

Capcitabine was given concurrently with radiotherapy (825 mg/m^2, twice daily). FOLFOX (fluorouracil, leucovorin, and oxaliplatin) regimen was given in the adjuvant setting for 4 months.

2.3.2.2 Chemotherapy

High risk stage II and stage III colon cancer patients received adjuvant chemotherapy. Risk factors for relapse included, poorly differentiated, tumor perforation or obstruction, number of retrieved lymph nodes <12, or lymphatic/vascular invasion. Chemotherapy regimens consisted of 6 cycles of 5 FU/Leucovorin, FOLFOX. All patients were followed up postoperatively at 3 months intervals for the first 2 years by history and clinical examination, laboratory investigations and imaging studies (including CT scan pelvis and abdomen).

Local-regional failure was defined as recurrence within the pelvis, including the tumor bed, regional lymph nodes, anastomosis, or perineal scar. Distant failure was indicated as disease recurrence detected in the liver, lung, brain, and other organs outside the pelvis. The recurrence of disease was confirmed by physical findings, radiological studies, endoscopic examination with biopsy, and surgery.

2.4 Tissue Handling

KRAS mutation detection.

2.4.1 DNA extraction

DNA was extracted from formalin fixed, paraffin-embedded tissues using the DNeasy Blood & Tissue Kit, (QIAGEN- Germany)**.** Small sections (not more than 25 mg) of paraffin-embedded tissue were used according to the kit guidelines. DNA concentration was defined with a range of 15- 150 ng**/**ul (the mean value of 70 ng/ul).

2.4.2 Real time PCR

The PCR reactions were carried out on LigtCycler instrument (Roche, Germany) using

PNA Clamp Technique for Detecting a Ki-ras2 Mutation.

2.4.2.1 Principle

Point mutations in codon 12 of the Ki-ras2 gene are associated with colon cancer [13]. The detection of a point mutation in the high background of wild-type cells is very difficult. Therefore, a quick and easy, reliable method of detecting single point mutations is preferred. An example for this is the Peptide Nucleic Acid (PNA) oligomers in combination with Hybridization Probes on the LightCycler Instrument.

2.4.2.2 PCR amplifications

The assay was based on the PCR amplification of a fragment from exon/intron 1 of the human *KRAS* gene (Fig. 1) and a subsequent melting curve analysis of hybridization probes. *KRAS* Mutations in codons 12 and/or 13 were recorded.

The reaction mix contained a wild type specific competitor oligonucleotide enriched all contained mutations in order to reduce the amplification of the wild type target. Since the competitor affected also the amplification of the mutated target, samples with low amounts of target DNA could be inhibited. Therefore we employed three different concentrations to obtain results from samples with different amounts of target. The observed melting temperature was dependent on the number of mismatches between probe and target. The kit contained pre-mixed reagents with three concentrations of competitor which were named CTRL (control), LOW (balanced low concentration) and HIGH (high concentration).

The HIGH reaction was adjusted to inhibit the wild type target completely but contained so much of competitor that very low target DNA samples could also be suppressed. The LOW reaction was adjusted to report most of the mutations, but also reported a wild type peak for samples which contained significant amounts of wild type target.

A control reaction was needed to report amplification for the samples which displayed a baseline in LOW and HIGH reactions and help to differentiate between fully suppressed low amount wild type samples in the LOW reaction from samples missing target DNA or complete PCR inhibition. Furthermore, the CTRL reaction was used to display a normal wild type (WT) specific melting temperature as reference.

Melting Curve Analysis – Controls:

The **HIGH** reagent should never report a wild type specific melting peak. Wild type samples displayed a baseline melting curve. Samples contained mutations, displayed only the mutation specific peak, even in presence of excess amounts of wild type DNA. The **CTRL** assay must report the wild type specific melting point (about 65℃). Samples contained significant amounts of mutated *KRAS* gene copies (>10-20%), displayed a second mutation specific peak. The data were read and compared **(CTRL**, **LOW** versus **HIGH** reactions) for each sample. The results from the controls were used for comparison; included the WT control from the LOW reaction to mark the wild type specific peak at 65℃ and to compare the peak magnitudes.

Typical Results and Interpretation: The claimed result was mutated or not mutated (Fig. 2).

- Samples displayed a **clear peak** in the **HIGH** reaction contained a mutated *KRAS* gene. The **LOW** reaction gave the same result or displayed two peaks from the wild type and the mutation.
- Samples showed a **peak >68℃** in the **HIGH** reaction contained the 12 Cystein mutation.
- Samples displayed **no peak** in the **HIGH** reaction but had a **clear peak** with any temperature lower than 65℃ and higher than 50℃ in the **LOW reaction** contained a mutation in codon 12 or codon 13 or both.
- Sample displayed **no peak** in the **HIGH** reaction and only a shoulder in the LOW reaction had to be considered as wild type.
- Peaks different from 65℃ (wild type) observed in the CTRL reaction which were not visible also in the **LOW** or **HIGH** reaction had to be reported as wild type.
- Samples which did not show any peak were non valid and should be repeated. Possible explanation is missing DNA or pipetting errors.
- Tm values and the shape of the melting curve in the **CTRL** reaction did not allow detection of the mutation unless it was a sample with > 50% content of mutation.

2.5 Acceptance Criteria (Control Samples) of the Lightcycler Protocol of *Kras* Mutation

13C (Cystein) LOW reaction: Sample must show a peak at about 56℃.

12C (Cystein) HIGH reaction: Sample must show a peak at about 68℃-70℃.

No template control (NTC): Sample must show baseline (no contamination).

Wild type (WT) LOW reaction: Sample must show a peak at about 64℃-65℃.

Low concentration of wild type (cWT) HIGH reaction: Sample must show baseline (max. 10% signal of WT in LOW).

2.6 Statistical Method

To determine relationships between clinic-pathological factors, patterns of failure and *KRAS* mutation status, the chi-square test was used. DFS was defined as the interval from enrollment of patients to the date of relapse or death from any cause or last follow up. Overall survival (OS) was defined as the interval from enrollment to the date of death from any cause or last follow up. DFS and OS rates were estimated by the Kaplan–Meier method [14]. The association between *KRAS* mutation status and survival was assessed univariately using Kaplan-Meir method. Log-rank test was used to detect the differences among survival curves. All *P* values, < 0.05 considered to be significant. All analyses were performed using the Statistical Package for Social Sciences software (version 18.0, SPSS, Chicago, IL).

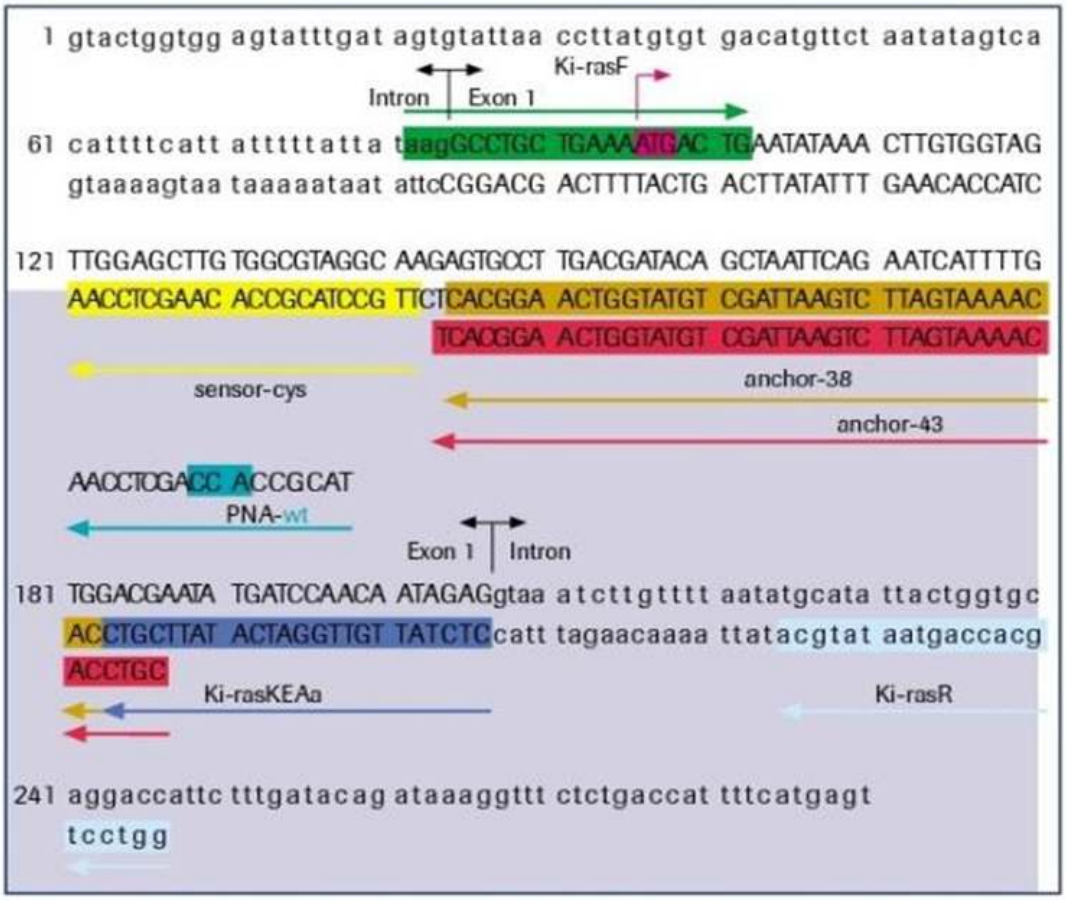

Fig. 1. Sequence and position of the Ki-ras2 target sequence (GenBank Accession # L00045)

Exon sequences (an exon 1 of the human cellular c-Ki-ras2 proto-oncogene) are typed in upper-case letters, intron sequences in lower-case letters. The PCR primers sense (green) and antisense (blue), as well as the Hybridization Probes sensor (yellow), anchor (red), the PNA oligomer, and intron/exon boundaries are indicated [13]

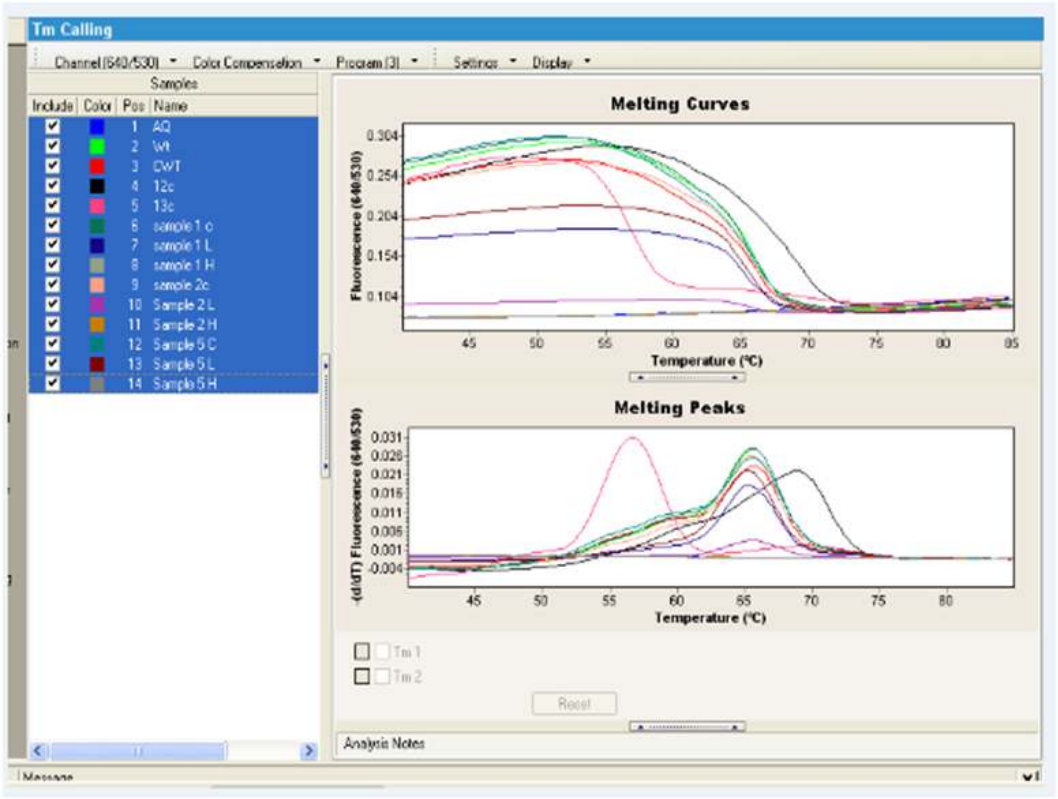

Fig. 2. *KRAS* mutation detection assay for 3 cases of colorectal cancer

KRAS mutation detection assay for 3 cases of colorectal cancer revealed that each sample represented by 3 reactions; control, low and high reaction. Also templates of wild type, control wild type, 12C mutation and 13C mutation are included in the assay

WT: Wild type; cWT: Low concentration wild type; 12C: 12 Cystein; 13C: 13 Cystein; Sample1c: Control of sample 1; H: High concentration reaction; L: Low concentration reaction; AQ: No template control

3. RESULTS

During the study period, a total of 79 histopathologically confirmed CRC cases were included. The number of males was 47 (59.5%) and the number of females was 32 (40.5%). The age of the patients ranged from 34 to 68 years (the mean age: 50.8 years ±11.0). Tumors were located in the colon in 37 patients (46.8%), in the rectum in 29 patients (36.7%) and in the rectosigmoid region in 13 patients (16.4%). Microscopically, 46 patients (58.2%) were classified as adenocarcinoma. Mucoid and signet ring carcinomas accounted for 27 (34.2%) and 6 (7.6%) patients respectively. Assessment of the nodal status of all patients revealed that 39 patients were node negative and 40 patients were node positive. According to TNM staging, 41 patients (51.9%) had stage III disease. The grade of differentiation of the tumors was assessed and there were 7 well differentiated, 24 moderately differentiated and 41 poorly differentiated. Fifty two tumors showed vascular invasion.

Genotyping revealed an overall mutation frequency of 31.6% (25 out of 79) in *KRAS* gene. Among 25 cases with *KRAS* mutated CRC, 68% (17 out of 25) had a mutation in codon 12 and 32% (8 out of 25) in codon 13. The most common mutations were glycine to aspartate on codon 12 (12D) and on codon 13 (13D) (Table 1).

3.1 Treatment Outcome

The period of follow-up of the patients ranged from 7 to 41 months with a median of 27 months. Treatment failed in 19 patients (24.0%) with median time of 17 months (range, 8 to 29 months). The patterns of treatment failure of the study group (79 patients) were, isolated local recurrence in 5 (6.3%) patients, isolated distant metastasis in 8 (10.1%), and local and distant

metastasis in 6 (7.6%). Among the 19 patients experienced recurrence, 4 patients (21.0%) had a reoperation and chemotherapy, 12 patients (63.1%) treated with palliative chemotherapy and radiotherapy to control symptoms, and 3 patients (15.8%) treated with best supportive care due to poor performance status.

After a median follow up period of 27 months, the 2-year OS and DFS were 87.9% and 77.5% respectively.

Clinicopathological characteristics of the study population according to *KRAS* mutation status were presented in (Table 2). There was no statistically significant association between clinicopathological variables (age, sex, tumour site, histology, grade, vascular invasion, tumor stage, nodal stage, or TNM stage) and *KRAS* mutation status.

Analysis of the correlation between *KRAS* mutation status and treatment outcome revealed a statistically significant increase in the rate of local recurrence (*P* =.002) and distant metastasis (*P* = .024) among patients with *KRAS* mutated compared with *KRAS* wild type CRC (Table 3).

There was statistically significant poorer 2-year OS (83.1% vs 90.7%, *P* = .04) and DFS (61.6% vs 86.2%, *P* = .01) in cases with *KRAS* mutated vs *KRAS* wild type CRC (Figs. 3 & 4). Codon 13 mutation was associated with statistically significant lower 2-year OS (62.5% vs 91.4%, *P* = .009) and DFS (46.9% vs 81.4%, *P* = .008) compared to cases without codon 13 *KRAS* mutation (Figs. 5 & 6). There was no statistically significant association between codon 12 mutation and DFS (*P* = .295) nor OS (*P* = .772) at 2-year.

4. DISCUSSION

In Egypt, colorectal cancer is the 6th cancer both in males and females representing 4.5% and 3.6% of the total cancers with age-standardized rates per 100,000 populations of 6.5% and 4.2% in males and females, respectively [15]. Gharbiah population based cancer registry, Egypt, identified 293 CRC cases between 2000 and 2002 and found that the median age was 53 years (range 21-81 years) with male predominance (1.3:1). The colon was the commonest site and the right and left sides were equally affected. Stage II disease was the commonest stage. Adenocarcinoma was the commonest histologic subtype. Low grade tumors were the commonest [15]. Our results revealed that the median age was 56 years (range 34-68 years) with male predominance (59.5%). Colon was the commonest site of tumor (46.8%). Adenocarcinoma was the most common histopathological tumor (58.2%) and the majority of the tumors were poorly differentiated (60.8%). Most of the patients were presented with stage III disease (51.9%). Northern Sweden Health Disease Study analyzed the prognostic value of *KRAS* in 197 cases with CRC and found that 56.3% were between 60-69 years of age with female predominance (56.9%). The commonest site of the tumor was rectum, constituted 39.6%. Non mucinous carcinoma was the commonest histologic subtype (80.6%) and the majority of the tumors were grade III-IV (46.2%). Most of the patients presented with advanced disease (46.2%) [16].

CRC is a highly heterogeneous disease with different genetic and molecular characteristics affecting intrinsic tumour aggressiveness, response to systemic treatment, and, hence, clinical outcome [17]. Thus, prognostic biomarkers are required to guide physicians for optimal management of the patients after curative resection of CRC. We analyzed the frequency of *KRAS* codon 12 and 13 mutations and determined their association with clinicopathologic variables and survival in 79 patients with localized CRC. In our study population, the mutational rate for *KRAS* was 31.6%, which was similar to the mutational rates reported in 3 large studies (30% and 37%) [18-20]. Liu et al. reported that mutation at codon 12 occurred in 69% (45/65) of cases [21]. This is in accordance with our study that revealed a mutation rate at codon 12 of 68% (17/25) of the cases. The most common alteration in our study was glycine to aspartate substitution on codon 12 (24.0%; n = 6 of 25). These findings were in line with previously published results; point mutations in codon 12 were the most common *KRAS* mutation in colorectal cancer [5,22–25].

In the present study, no statistically significant correlations were noted between the clinicopathological variables and *KRAS* mutation status. However, Bazan et al. [26], showed an association between the *KRAS* mutation in codon 13 and advanced clinical stages; the presence of lymph node metastasis and high S-phase fraction, while *KRAS* mutation at codon 12 was associated with mucinous histotype. Yoon et al. [2] found that, compared to wild type, *KRAS* mutations were significantly associated with older

age and female sex, primarily due to mutations in codon 12, and did not differ by T stage or number of positive nodes. Compared to *KRAS* wild type, codon 12 and 13 mutations were each associated with proximal *vs* distal tumor site within the colon (*P*<.0001). Codon 12 and 13 mutations were associated with low and high grade histology, respectively, in primary tumors. Other reports did not find correlation between the incidence of *KRAS* mutations and clinical features [19,21,25,27].

Several studies did not find an association between *KRAS* and treatment outcomes [28-34]. Our study however, demonstrated significant increase in rate of local recurrence (*P*= .002), distant metastasis (*P*= .024) and local and distant metastasis (*P*= .05).

El-Serafi et al. [35] found that, the frequency of *KRAS* overexpression and mutations (48.9% and 41.1%, respectively), had a significant correlation with reduced OS. Yoon et al. [2] reported, tumors carried *KRAS* codon 12 or 13 mutations experienced a 52% or 36% higher relative risk, respectively, of colon cancer recurrence or any cause death independent of clinicopathological variables or mismatch repair status. We found a statistically significant poorer DFS and OS of patients with *KRAS* mutated CRC compared to those with *KRAS* wild type. Our result were comparable to that reported by Phipps et al., who stated that the presence of a somatic *KRAS* mutation was associated with statistically significant poorer survival, specifically in those without distant-stage disease [36]. Other studies have also indicated a poorer prognosis in patients with *KRAS*-mutated CRC [37-40]. On the other hand several studies have found no association of *KRAS* mutated tumors and survival [28-34].

Table 1. Distribution of the 25 mutations of *KRAS* gene[a]

Mutated codon	Types of mutation	Amino acid	No. of mutations (%) out of 25 cases
12	12D	Aspartate	6 (24.0)
	12V	Valine	5 (6.3)
	12C	Cysteine	3 (3.8)
	12R	Arginine	2 (2.5)
	12S	Serine	1 (1.3)
Total			17 (68.0)
13	13D	Aspartate	5 (6.3)
	13C	Cysteine	3 (3.8)
Total			8 (32.0)

[a]*Data are given as number (percentage). D: Glycine to Aspartate; V: Glycine to Valine; C: Glycine to Cystein; R: Glycine to Arginine; S: Glycine to serine*

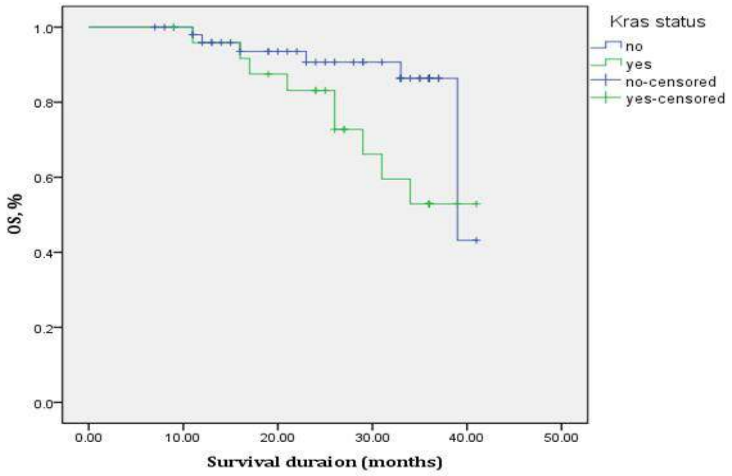

Fig. 3. Overall survival according to *KRAS* mutation status

Kaplan-Meir analysis illustrated significant decrease in 2-year overall survival (83% vs 70%, P=.04) in 79 colorectal cancer cases with KRAS mutation

Table 2. Clinicopathological characteristics by *KRAS* mutation status in 79 cases with colorectal cancer[a]

Variables		*KRAS* wild-type (N= 54)(%)	*KRAS* mutated (N= 25)(%)	*P*-value[b]
Age at diagnosis (Mean ± SD)	51.3 ± 10.9	52.0±11.0	49.9±10.8	.435
Age group				
	<40 (n=21)	13 (61.9)	8 (38.1)	.851
	40- 49 (n=18)	12 (66.7)	6 (33.3)	
	50-59 (n=23)	17 (73.9)	6 (26.1)	
	≥ 60 (n=17)	12 (70.6)	5 (29.4)	
Sex	Male (n=47)	32 (68.1)	15 (31.9)	.950
	Female (n=32)	22 (68.8)	10 (31.3)	
Performance Status	0 (n = 22)	18 (81.8)	4 (18.2)	.110
	1 (n = 57)	36 (63.2)	21 (36.8)	
Site of tumor	Colon (n=37)	27 (73.0)	10 (27.0)	.117
	Rectum (n=29)	16 (55.2)	13 (44.8)	
	Rectosigmoid (n=13)	11 (84.6)	2 (15.4)	
Histologic types	Adenocarcinoma (n=46)	31 (67.4)	15 (32.6)	.962
	Mucoid carcinoma (n=27)	19 (70.4)	8 (29.6)	
	Signet ring carcinoma (n=6)	4 (66.7)	2 (33.3)	
T- stage	T2 (n=10)	8 (80.0)	2 (20.0)	.414
	T3 (n=53)	37 (69.8)	16 (30.2)	
	T4 (n=16)	9 (56.3)	7 (43.8)	
N- stage	N0 (n=39)	27 (69.2)	12 (30.8)	.829
	N1 (n=30)	21 (70.0)	9 (30.0)	
	N2 (n=10)	6 (60.0)	4 (40.0)	
TNM	I (n=4)	3 (75.0)	1 (25.0)	.956
Stage	II (n=34)	23 (67.6)	11 (32.4)	
	III (n=41)	28 (68.3)	13 (31.7)	
Grading	G1 (n=7)	4 (57.1)	3 (42.9)	.750
	G2 (n=24)	16 (66.7)	8 (33.3)	
	G3 (n=48)	34 (70.8)	14 (29.2)	
Vascular invasion	No (n=52)	39 (75.0)	13 (25)	.078
	Yes (n=27)	15 (55.6)	12 (44.4)	

[a]*Data are given as number (percentage);*
[b]*Comparing cases with KRAS mutated to cases with wild type colorectal cancer*

In our study, mutation of *KRAS* in codon 12 was not significantly associated with reduced DFS or OS. However, *KRAS* codon 13 mutation was significantly associated with worse DFS (46.9% vs 81.4%) and OS (62.5% vs 91.4%) compared with patients whose tumors were wild type for *KRAS.* Wangefjord et al. [18], reported similar survival for patients with *KRAS* wild-type and codon 12-mutated tumors, while patients with tumors harboring a *KRAS* codon 13 mutation had a significantly reduced Cancer specific survival (HR = 1.94; 95% CI = 1.18–3.19) in unadjusted, but not in adjusted analysis. Imamura et al., observed that specific *KRAS* mutations were significantly associated with patient survival in Kaplan-Meier analysis (log-rank *P* = .0014) [41]. Bazan et al. [26] showed that in multivariate analysis, mutation in codon 13 was an independent prognostic factor for poor DFS and OS. Samowitz et al. [28], observed in a large population based study of codon 13 G →A mutation was associated with reduced survival. Imamura et al. [40], reported that *KRAS* codon 12 mutations, particularly the G12V mutation, but not *KRAS* codon 13 mutations were associated with poorer survival. Andreyev et al. [42], reported that the presence of a somatic *KRAS* mutation was associated with statistically significantly poorer disease-free and overall survival among patients with Dukes' C CRC and

confined to those with *KRAS* G12V mutation. On the other hand, several reports have found significant correlation between *KRAS* codon 12 mutations and aggressive forms of disease with reduced survival rates [19,26,43]. The reasons for inconsistency of our result compared to other literature might be the small sample size of our study population, and variations in the distribution of patients and tumor characteristics.

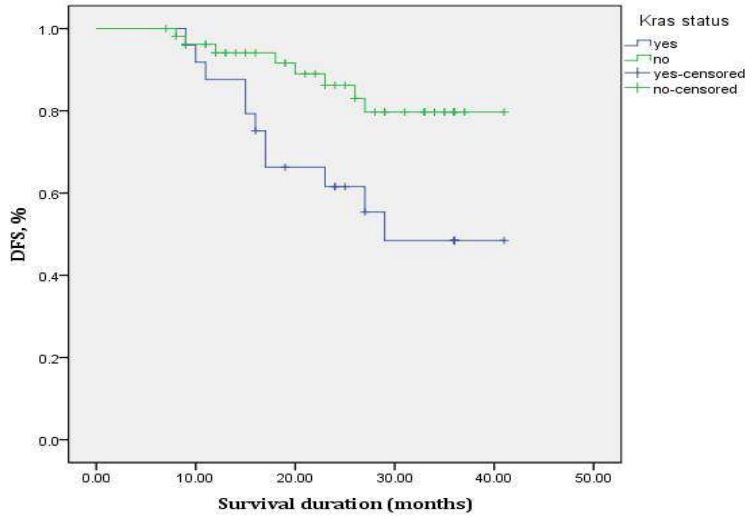

Fig. 4. Disease free survival according to *KRAS* mutation status

Kaplan-Meir analysis illustrated significant decrease in 2-year disease free survival (61.6% vs 86.2%, P = .01) in 79 colorectal cancer cases with KRAS mutation

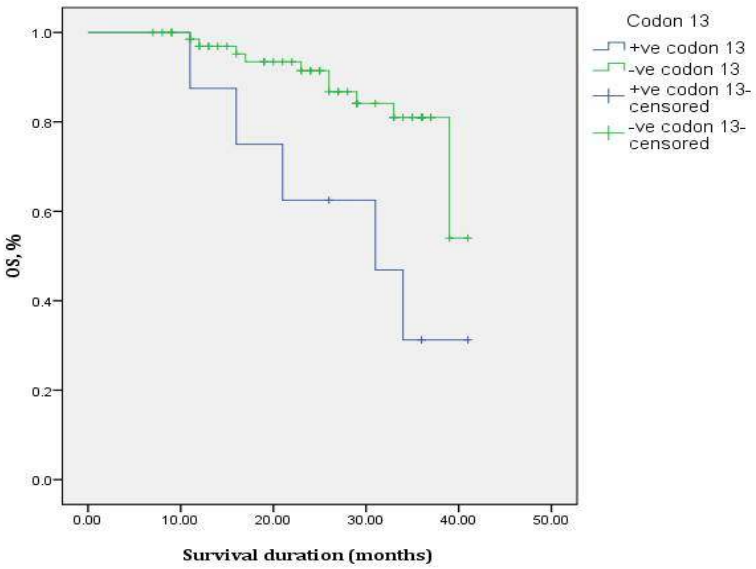

Fig. 5. Association between overall survival and mutation in codon 13

Mutation in codon 13 was associated with statistically significant lower 2-year overall survival (62.5% vs 91.4%, P=.009) compared to cases without codon 13 KRAS mutation. The symbol "-ve" means negative value and the symbol "+ve" means positive value

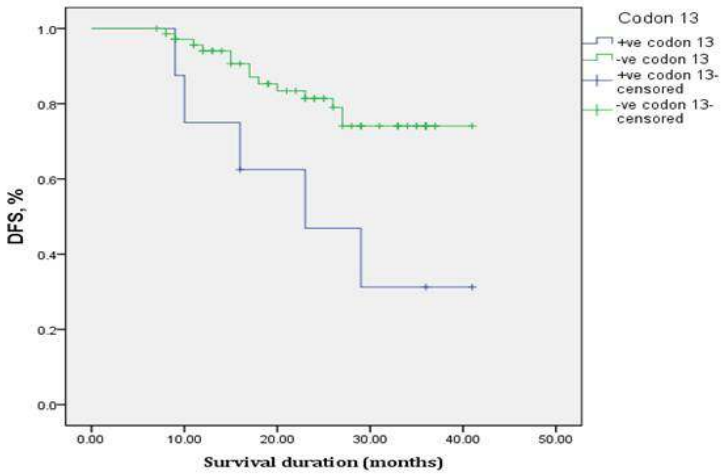

Fig. 6. Association between disease free survival and codon 13

Codon 13 mutation was associated with statistically significant lower 2-year disease free survival (46.9% vs 81.4%, P=.008) compared to cases without codon 13 KRAS mutation. The symbol "-ve" means negative value and the symbol "+ve" means positive value

Table 3. Pattern of treatment failure according to *KRAS* mutation status in 79 cases with colorectal cancer[a]

Pattern of treatment failure		KRAS mutation status		Total (%)	*P*-value[b]
		No no. (%)	Yes no. (%)		
Local recurrence	No	51 (75)	17 (25)	68 (100)	.002
	Yes	3 (27.3)	8 (72.7)	11 (100)	
Distant metastasis	No	48 (73.8)	17 (26)	65 (100)	.024
	Yes	6 (42.9)	8 (57.1)	14 (100)	

[a]*Data are given as number (percentage);*

[b]*Comparing cases with KRAS mutation to cases with wild type colorectal cancer*

The potential limitations of this study were the small number of patients and this study included only patients who were evaluated and treated at South Egypt cancer Instiute, which may not reflect the whole population in Egypt. However, despite this limitation, the study has provided local data that need to be addressed in order to help to deliver optimal care and management of patients with *KRAS* mutated CRC.

5. CONCLUSION

Our study confirms previous reports that *KRAS* mutated CRC was not associated with clinicopathological variables. *KRAS* mutations were associated with statistically significant increase in all types of recurrences and this should be considered in predicting the clinical outcome of the patients. Our study demonstrated that *KRAS* tumor mutation status has prognostic value for DFS and OS in patients with stage II and III colorectal cancer treated with adjuvant chemotherapy or chemoradiotherapy. *KRAS* codon 13 mutations were independent prognostic factor for both DFS and OS, while KRAS codon 12 mutations were not significantly associated with reduced DFS or OS. The prognostic significance regarding survival and treatment outcome of *KRAS* mutations in resected CRC might form the basis for more specific adjuvant therapy to improve outcome and survival.

CONSENT

Written informed consent was obtained from all patients included in this study.

ETHICAL APPROVAL

The study was approved by the institutional ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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