Clinics in Oncology

Research Article
Published: 07 May, 2021

CEA and CA19-9 Levels and *KRAS* Mutation Status as Biomarkers for Colorectal Cancer

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Abstract

Aim: We aimed to investigate the associations among *KRAS* mutation, carcinoembryonic Antigen (CEA), and Cancer Antigen 19-9 (CA19-9) levels, and the clinicopathological features of Colorectal Cancer (CRC) patients.

Methods: We conducted a retrospective cohort study, involving 183 CRC patients, at Princess Norah Oncology Center (PNOC), King Abdulaziz Medical City, Jeddah, Saudi Arabia, from 2012 to 2015.

Result: A strong association was identified between CA19-9 levels and *KRAS* mutational status and between CA19-9 and CEA levels, in CRC patients (p=0.001 and p<0.0001, respectively). Large tumor size was associated with abnormally high CEA levels (p=0.029). Furthermore, young patients (<45 years) primarily expressed wild-type *KRAS* (p=0.013). The levels of CA19-9 and CEA were found to increase with increasing tumor stage (p<0.0001 and p=0.001, respectively). Mutated KRAS and abnormal CEA levels were associated with lymph node involvement (p=0.054 and p=0.014, respectively). The most common sites of distant metastases were the liver and lung in CRC patients with abnormal serum CA19-9 and CEA levels. Kaplan-Meier survival analysis showed that patients with high CA19-9 levels have shorter survival times than patients with normal CA19-9 levels (p<0.0001). Significant effects of CEA19-9 levels on the detection of *KRAS* mutations were analyzed by multivariate regression analysis (p<0.001).

Conclusion: This study provided new insights into the important roles played by CA19-9 and CEA during CRC progression and suggested that they may serve as useful biomarkers in CRC management.

Strengths and limitations of this study

- This is one of very few studies that assessed biomarkers of CRC among the Saudi population.
- This is one of the first studies to determine the independent predictors of the *KRAS* mutation patients in Saudi Arabia. The results of this study demonstrate that CEA and CA19-9 levels can be used to indicate *KRAS* mutation status and can be used as surrogate biomarkers.
- The sample size was limited to a single centre. A Larger sample size ensures adequate statistical power to detect even a small effect of interest.
- The study is retrospective, so we were not able to obtain more information from the participants.

Keywords: *KRAS*; Mutation; CEA; CA19-9; Hemoglobin; Colorectal cancer

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Received Date: *11 Apr 2021*
Accepted Date: *04 May 2021*
Published Date: *07 May 2021*

Citation:
Subki AH, Alghamdi TA, Butt NS, Alqazlan MS, Alkahtani AM, Aziz MA, et al. CEA and CA19-9 Levels and KRAS Mutation Status as Biomarkers for Colorectal Cancer. Clin Oncol. 2021; 6: 1802.

Background

Colorectal Cancer (CRC) is a major health problem, worldwide. CRC is the third most common type of cancer in both men and women, and it is the third-leading cause of cancer-related deaths. In 1975, the CRC death rate was 28/100,000, compared with 14/100,000 in 2015. This decline can

be attributed to improved treatments and early detection measures [1]. The Saudi Cancer Registry reported 1,347 new cases of CRC in 2014, including 753 men and 594 women, with a male:female ratio of 127:100 [2]. The Saudi Arabian Age-Standardized Rate (ASR) was 10.6/100,000 in men and 8.2/100,000 in women. The Saudi Arabian ASR per 100,000 populations for CRC is lower than that for Kuwait, Qatar, and Bahrain but higher than that for the United Arab Emirates and Oman [2-4].

Cetuximab and panitumumab are anti-Epidermal Growth Factor Receptor (EGFR)-targeted antibodies, used for the treatment of Metastatic CRC (mCRC) [5-7]. EGFR failed to serve as a predictor of the therapeutic response to these drugs [8,9]; however, the mutation status of the Kirsten RAS (*KRAS*) proto-oncogene is the most well-established predictive biomarker for CRC response [10,11]. *KRAS* mutations are not only predictors of the response to anti-EGFR drug treatment but are also associated with significantly worse survival than wild-type *KRAS* [12,13].

Carcinoembryonic Antigen (CEA) is a glycoprotein, containing 30% to 70% carbohydrates. CEA is significantly expressed by the large intestine during the embryonic stage and in CRC [14,15]. Increased CEA levels have been observed in the digestive tract, lungs, and breast cancers. Metastatic tumors in the liver and pancreas and medullary carcinoma of the thyroid can release CEA into the blood circulation, increasing serum CEA levels [16,17]. Non-malignant disorders might also increase CEA levels, such as liver disease, active inflammatory bowel disease, and aging. Higher serum CEA levels have been identified in heavy smokers compared with the levels in healthy, non-smoking individuals. However, CEA can be found in small amounts in the large intestine of healthy adults and circulating in the blood of normal individuals [18].

Carbohydrate antigen (CA19-9) is a glycoprotein that contains sialylated Lewis-a, a blood group antigen [19]. CA19-9 is normally produced by gastric, pancreatic, biliary, and colonic epithelial cells. CA19-9 is also expressed by cancer cells and is involved in the adhesion of tumor and endothelial cells. CA19-9 is used as a tumor marker in gynecological, pancreatic, hepatobiliary, and CRC [20,21] and can indicate the presence of progressive malignant disease and poor response to treatment when serum levels are elevated. CA19-9 can be used to detect the recurrence of cancer after treatment, even before other clinical findings and radiographs. A decrease in CA19-9 levels may indicate a good treatment response and favorable prognosis [22-24].

In our retrospective study, we aimed to investigate the associations among *KRAS* mutations, CA19-9, CEA, and the clinicopathological features in CRC patients. We found that CA19-9 and CEA are associated with clinical indicators, such as tumor stage and survival. This study provided new insights into the important roles played by CA19-9 and CEA in CRC progression and suggested their potential use as biomarkers for CRC management.

Methods

Patient and public involvement

A retrospective cohort study was conducted to investigate the association between *KRAS* mutation, hemoglobin, CEA, and CA19-9 levels, and the clinicopathological features of CRC patients (age, gender, tumor size and stage, and response to therapy). We reviewed the paper-based and electronic medical records of CRC patients at the Princess Norah Oncology Center (PNOC), King Abdullah Medical City, Jeddah, Saudi Arabia, from 2012 to 2015. All patients ≥ 18 years, who were diagnosed at or referred to PNOC with biopsy-confirmed CRC, regardless of nationality and gender, were included (n=183). Patients younger than 18 years, who did not undergo KRAS mutation testing, and those missing data from their medical records were excluded from the study (n=52).

Cohort description

Patient data were retrieved from electronic hospital information systems and paper-based files, including gender, age, and nationality, date of birth, survival status, KRAS mutation, CA19-9, CEA, and hemoglobin status. We also extracted data regarding the pathological features of CRC, tumor type (primary/secondary), tumor location, tumor size, tumor stage, tumor grade, metastatic sites, Tumor Node Metastasis (TNM) staging, and date of tumor recurrence. Laboratory tests results at the time of diagnosis were obtained and incorporated into the study. Information regarding the type of treatment received and the response to treatment were also recorded and analyzed.

KRAS mutation status analysis

Formalin-fixed, paraffin-embedded tissue samples that were obtained from surgery were collected and sent to lab 21, in Cambridge, United Kingdom, where real-time Polymerase Reaction Assay (PCR) was performed. Codon 12, Codon 13, and Codon 61 were used to analyze *KRAS* mutations. The following mutations were tested: p.(Gly12Phe), p.(Gly12Cys), p.(Gly12Ser), p.(Gly12Arg), p.(Gly12Val), p.(Gly12Asp), p.(Gly12Ala), p.(Gly13Ile), p.(Gly13Cys), p.(Gly13Arg), p.(Gly13Ser), p.(Gly13Asp), p.(Gly13Ala), p.(Gly13Val), p.(Gln61Lys), p.(Gln61Glue), p.(Gln61pro), p.(Gln61Arg), p.(Gln61Leu), and p.(Gln61His). Samples were tested using the Cobas® *KRAS* Mutation Kit, which is marked under the European IVD Directive 98/79/EC (F. Hoffmann-La Roche, Basel, Switzerland) [25].

Statistical methods

Data were described as frequencies and percentages, for categorical variables. Associations among *KRAS* mutation, CA19-9, CEA, and various other study variables were assessed using the Chi-square and Fisher's exact tests. A binary logistic regression was conducted to examine whether CA19-9, CEA, metastasis, gender, tumor location, hemoglobin, or age had a significant role in the prediction of KRAS mutation. Kaplan-Meier survival curves and the log-rank test were used to analyze the survival distribution, based on KRAS mutation, CEA, and CA19-9 status. Statistical Package for Social Sciences (SPSS®), version 24 was used for data analysis.

Results

Clinical characteristics and serum levels of CEA and CA19-9

Our study included 183 patients diagnosed with CRC. The mean age of participants was 58.01 years, with a standard deviation of 12.7 years, and a median age of 57 years. The majority of participants were older than 45 years (85.8%; n=157) and male (55.7%; n=102), and almost all were Saudi nationals (98.9%; n=181). CRC tumors were most commonly diagnosed at stage 4 (51.2%; n=89) and sized between 4 cm to 6 cm (53.2%; n=75). The frequency distributions of the CA19-9 and CEA levels are shown in Figure supplemental A and supplemental B, respectively. Among 143 analyzed patients, 37 (25.9%) had elevated CA19-9 levels (normal value is <37 IU/ml).

Table 1: Association among mutations, CA19-9 and CEA levels, and other study variables.

		KRAS					CA19-9					CEA				
		WT		Mutated		*P*-value	Normal		Abnormal		*P*-value	Normal		Abnormal		*P*-value
		n	(%)	n	(%)		n	(%)	n	(%)		n	(%)	n	(%)	
Gender	Male	62	-61	40	-39	0.476	61	-75	20	-24.7	0.712	47	-50	47	-50	0.51
	Female	45	-56	36	-44		45	-73	17	-27.4		36	-45	44	-55	
Age	<45 Years	21	-81	5	-19	0.013*	16	-76	5	-23.8	1	14	-54	12	-46.2	0.496
	≥ 45 Years	86	-55	71	-45		90	-74	32	-26.2		69	-47	79	-53.4	
KRAS	Mutated						34	-60	23	-40.4	0.001*	32	-45	39	-54.9	0.564
	WT						72	-84	14	-16.3		51	-50	52	-50.5	
Carbohydrate antigen 19-9 (CA19-9)	0–37 IU/ml	72	-68	34	-32	0.001*						60	-58	43	-41.7	<0.0001*
	>37 IU/ml	14	-38	23	-62							5	-14	32	-86.5	
Carcinoembryonic antigen (CEA)	0.6–4 ng/ ml	51	-61	32	-39	0.564	60	-92	5	-7.7	0.000*					
	>4 ng/ml	52	-57	39	-43		43	-57	32	-42.7						
Hemoglobin levels	Normal	51	-61	32	-39	0.457	48	-73	18	-27.3	0.724	43	-52	39	-47.6	0.237
	Abnormal	56	-56	44	-44		58	-75	19	-24.7		40	-44	52	-56.5	
Primary Tumor	No	1	-50	1	-50	0.999[b]	2	-100	0	0	0.999[b]	0	0	2	-100	0.999[b]
	Yes	106	-59	75	-41		104	-74	37	-26.2		83	-48	89	-51.7	
Recurring Tumor	No	103	-58	74	-42	0.999[b]	102	-74	36	-26.1	0.999[b]	79	-47	89	-53	0.427[b]
	Yes	4	-67	2	-33		4	-80	1	-20		4	-67	2	-33.3	
Cancer morphology	Adenocarcinoma	96	-58	69	-42	0.480[b]	98	-75	32	-24.6	0.280[b]	77	-49	80	-51	0.481[b]
	Mucinous Adenocarcinoma	8	-62	5	-39		6	-60	4	-40		5	-39	8	-61.5	
	Signet Ring Cell Carcinoma	2	-100	0	0		0	0	0	0		0	0	1	-100	
Cancer grade	Well-differentiated	11	-58	8	-42	0.838	12	-86	2	-14.3	0.489	9	-50	9	-50	0.837
	Moderately-differentiated	79	-59	55	-41		78	-74	28	-26.4		64	-49	66	-50.8	
	poorly-differentiated	13	-62	8	-38		10	-67	5	-33.3		8	-42	11	-57.9	
Size Categories (Cm)	0–3	21	-64	12	-36	0.056*	18	-72	7	-28		22	-67	11	-33.3	0.029*
	4–6	47	-63	28	-37		51	-85	9	-15	0.198	39	-53	34	-46.6	
	>6	13	-39	20	-61		16	-70	7	-30.4		10	-33	20	-66.7	
Tumor **AJCC** Stage	Stage 1	8	-80	2	-20	0.348[b]	7	-100	0	0	<0.0001*	9	-90	1	-10	0.001*
	Stage 2	24	-60	16	-40		26	-93	2	-7.1		21	-55	17	-44.7	
	Stage 3	21	-60	14	-40		30	-94	2	-6.3		20	-59	14	-41.2	
	Stage 4	51	-57	38	-43		37	-53	33	-47.1		28	-33	56	-66.7	
Tumor TNM stage																
Primary tumor invasion (T)	T1	6	-86	1	-14	0.144	5	-100	0	0 (0)	0.323	5	-71	2	-28.6	0.041*
	T2	7	-70	3	-30		7	-88	1	-12.5		8	-80	2	-20	
	T3	38	-54	33	-47		47	-86	8	-14.5		37	-55	30	-44.8	
	T4	25	-71	10	-29		18	-72	7	-28		12	-35	22	-64.7	
Lymph node status	N0	41	-66	21	-34	0.054*	40	-89	5	-11	0.211	35	-60	23	-39.7	0.014*
	N1	30	-64	17	-36		31	-80	8	-20.5		24	-52	22	-47.8	
	N2	4	-31	9	-69		6	-67	3	-33.3		2	-15	11	-84.6	
Distant metastases	No (M0)	54	-64	30	-36	0.153	65	-96	3	-4.4	<0.0001*	49	-61	32	-39.5	0.003*
	Yes (M1)	27	-52	25	-48		19	-50	19	-50		17	-34	33	-66	

* The Chi-square statistic is significant at the 0.05 level

[b] More than 20% of cells have expected cell counts less than 5 Fisher's Exact test is used

Moreover, among 174 analyzed patients, 91 (52.3%) had elevated CEA levels (≥ 4 ng/ml). Hemoglobin levels were abnormal in 54.6% (n=100) of CRC patients, and 41.5% (n=76) had *KRAS* mutations. Response to treatment was almost equally distributed, with 32% (n=49), 33.3% (n=51), and 34.7% (n=53) of patients treated for CRC reporting stable, partial, and progressive disease, respectively (Supplemental Table 1).

Most tumors in our study were distributed on the left-sided colon (83%; n=152). The right-sided colon had 23 cases (12.6%), whereas

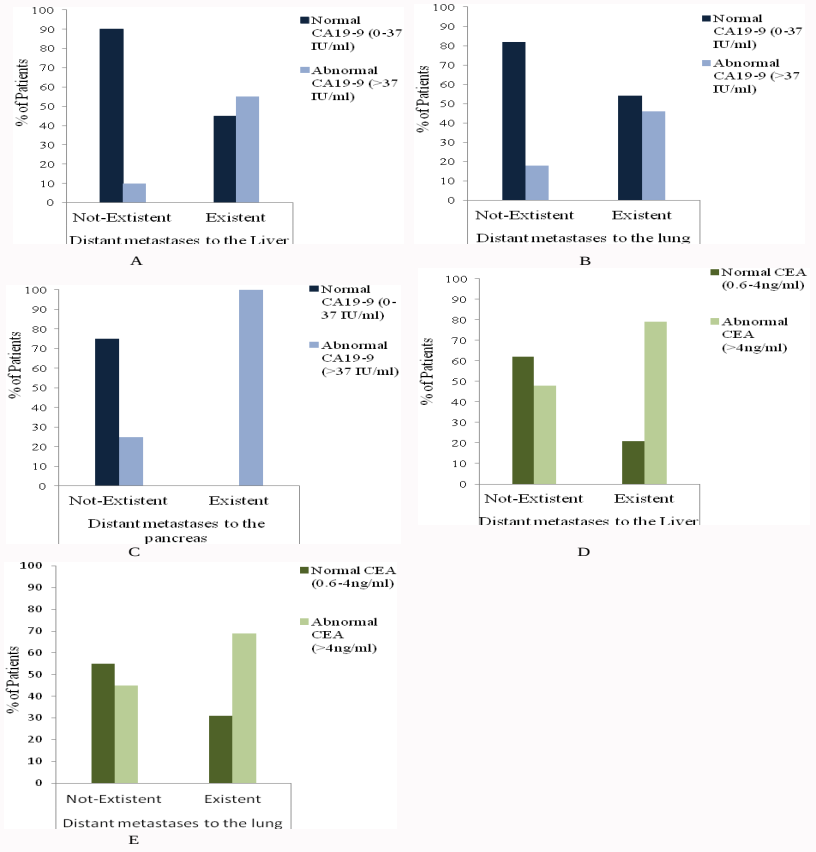

Figure 1: CA19-9 and CEA Serum level correlate with site of distant metastasis in CRC. CA19-9 serum level correlates with distant metastases to the (A) liver (p<0.0001), (B) lung (p<0.0001) and (C) pancreas (p=0.016). Abnormal CEA serum level is high in CRC patients who have clinically detectable (D) liver metastases (p<0.0001) and (E) lung metastases (p=0.007).

the transverse colon had only 8 cases, constituting merely 4.4% of the overall CRC diagnoses in our study. Most tumors were found in the area extending from the sigmoid to the rectum, especially the sigmoid colon (28.4%; n=52), followed by the rectosigmoid junction (21.9%; n=40) and the rectum (20.2%; n=37) (Supplemental Table 2).

Associations among *KRAS* mutation, CA19-9 and CEA levels, and clinical parameters

The associations among KRAS mutation, CA19-9 and CEA levels, and clinical parameters were investigated (Table 1). A strong association was identified among CA19-9 levels, KRAS mutation, and CEA levels, in which 62.2% of CRC patients with *KRAS* mutation revealed abnormally high CA19-9 levels (p=0.001) and 86.5% of CRC patients abnormally high CEA levels also presented abnormally high CA19-9 levels (p<0.0001). Large tumors, sized >6 cm, were associated with *KRAS* mutation (p=0.056) and abnormal CEA levels (p=0.029). Furthermore, patient age was associated with *KRAS* mutation, with 80.8% of young patients (<45 years) expressing wild-type *KRAS* and only 19.2% of young patients expressed mutated *KRAS* (p=0.013). No significant associations were observed between *KRAS* mutation, or hemoglobin, CA19-9, and CEA levels and other demographic and clinical parameters (gender, nationality, tumor grade, tumor morphology, and primary tumor site).

Abnormal levels of CA19-9 and CEA in CRC patients were associated with histopathological AJCC staging histopathological and TNM staging

While *KRAS* mutation did not correlate with AJCC tumor stage, levels of CA19-9 and CEA were found to increase with increasing tumor stage. Patients diagnosed with stage 1 tumors displayed normal CA19-9 levels (n=7, 100%), whereas abnormal CA19-9 levels were observed in 47.1% (n=33) of patients with stage 4 tumors (p<0.0001). Similarly, CEA levels increased abnormally in 66.7% (n=56) of patients with stage 4 tumors, whereas 90% (n=9) of patients with stage 1 tumors expressed normal CEA levels (p=0.001). *KRAS* mutation and CA19-9 and CEA levels were assessed using the TNM staging system, which is used to determine disease severity based on the degrees of tumor invasiveness (T1, T2, T3, and T4), regional lymph node involvement (N0, N1, and N2), and distant metastasis (M0 and M1). A significant difference in CEA levels was observed among the T1, T2, T3, and T4 groups (p=0.041). When samples in the N0, N1, and N2 groups were compared, mutated KRAS and abnormal levels of CEA were found to be associated with lymph node involvement (p=0.054 and p=0.014, respectively). Furthermore, CRC patients with distance metastasis showed abnormally high levels of CA19-9 and CEA. Among CRC patients with distant metastasis, 50% (n=19) presented with abnormal CA19-9 level (p<0.0001), and 66% (n=33) presented with abnormal CEA levels (p=0.003). The site of distant metastasis was also evaluated (Supplemental Table 3). In CRC patients with abnormal CA19-9 levels, the most common sites of distant metastasis were the liver (p<0.0001), lung (p<0.0001), and pancreas (p=0.016, Figures 1A-1C). In addition, the liver and lung were the only two distant metastasis sites correlated with CRC patients with abnormal CEA levels (p<0.0001 and p=0.007, respectively, Figure 1D and 1E). Distant metastases to all other sites were not significantly associated with KRAS mutation or CA19-9 and CEA levels.

Table 2: Logistic regression results, showing the ability of CA19-9 levels, CEA levels, metastasis, gender, tumor location, hemoglobin, and age to predict KRAS mutation.

Variable	*B*	*SE*	95.0% CI	χ^2	*p*	*OR*
(Intercept)	−1.17	1.33	[−3.77, 1.44]	0.77	0.38	
CA19-9 (0–37 IU/ml)	−1.86	0.54	[−2.92, −0.79]	11.72	<0.001	0.16
CEA (>4 ng/ml)	−0.45	0.42	[−1.28, 0.38]	1.11	0.293	0.64
Metastasis (Yes)	−0.48	0.45	[−1.37, 0.40]	1.15	0.284	0.62
Gender (Female)	0.4	0.38	[−0.36, 1.15]	1.06	0.304	1.49
Location of Tumor (Left-Sided Colon)	1.14	0.63	[−0.08, 2.37]	3.34	0.067	3.14
Location of Tumor (Unspecified/ Overlapping)	1.64	1.19	[−0.70, 3.98]	1.89	0.169	5.17
HB (Normal)	−0.57	0.39	[−1.33, 0.20]	2.11	0.147	0.57
Age	0.03	0.02	[−0.00, 0.06]	3.08	0.079	1.03

Note: $\chi^2(8)=19.32$, p=0.013, McFadden $R^2=0.10$

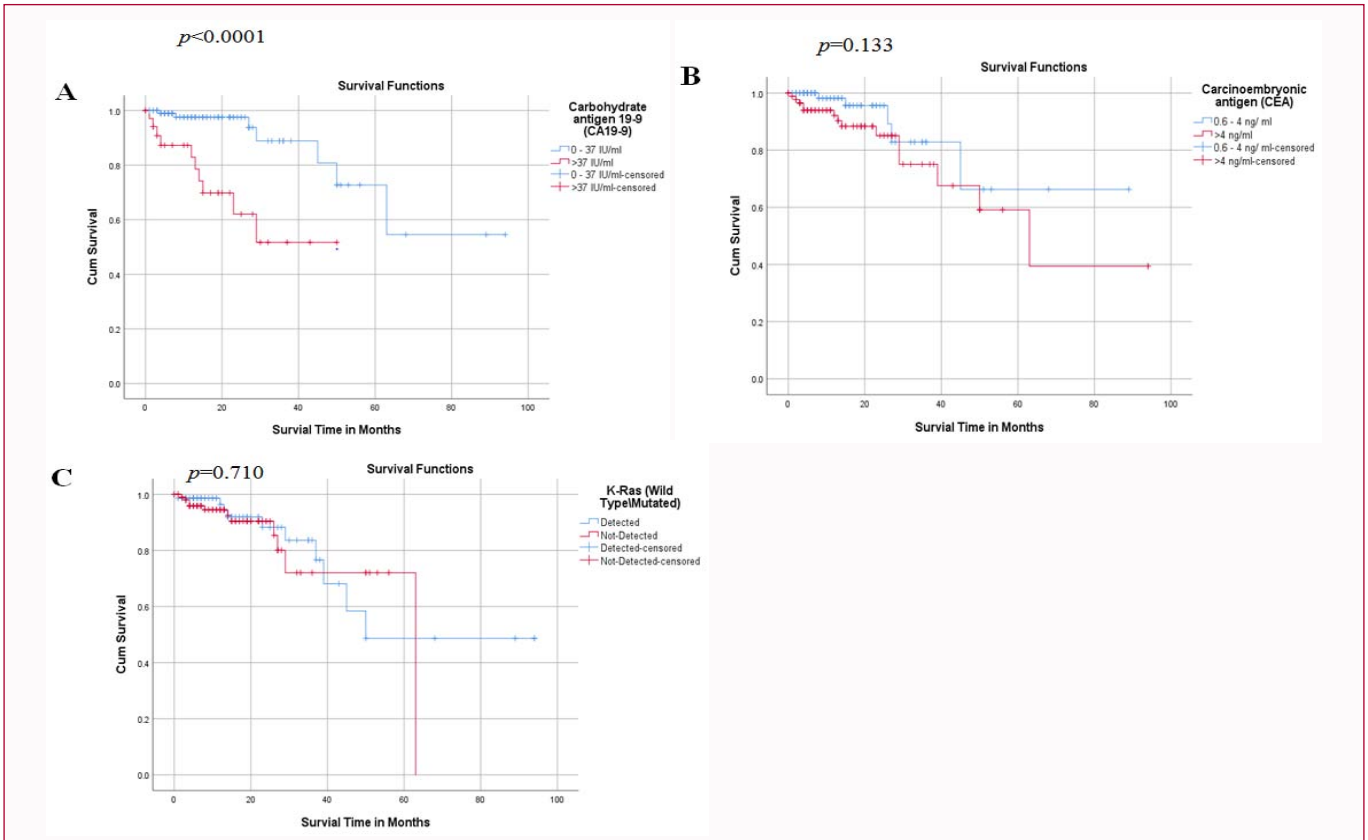

Figure 2: Kaplan Meier Survival Curves of CRC by CA19-9, CEA and KRAS: (A) Patients with abnormal CA19-9 serum level have a shorter survival time compared to patients with normal CA19-9 serum level (p=0.000). (B) CEA serum level and (C) KRAS gene mutation status was not associated with overall survival.

Association between CA19-9 serum levels and overall survival (OS) outcomes in CRC patients

Associations among KRAS mutation, CA19-9 and CEA serum levels, and Overall Survival (OS) were evaluated by Kaplan-Meier plots. The Kaplan-Meier survival plot showed a strong correlation between CA19-9 levels and survival (p<0.0001, Figure 2A). The correlation indicated that patients with abnormal CA19-9 levels have shorter survival times than patients with normal CA19-9 levels. The median survival time for patients with abnormal CA19-9 levels was 664 days, whereas the median survival time for patients with normal CA19-9 levels was 1,706 days, more than twice as long. No associations were detected between OS and *KRAS* mutation or between OS and CEA serum levels (Figure 2B and 2C, respectively).

Multivariate regression analysis, showing the significant effect of CEA*19-9* levels for the detection of *KRAS* Mutation

The model was evaluated based on an alpha of 0.05. The overall model was significant ($\chi^2(8)=19.32$, p=0.013), suggesting that CA19-9 and CEA levels, metastasis, gender, tumor location, hemoglobin, and age had significant effects on the odds of observing KRAS mutation (Table 2).

The regression coefficient for CA19-9 levels was significant [B= −1.86, odds ratio (OR)=0.16, p<0.001], indicating that a respondent with CA19-9 levels between 0 IU/ml to 37 IU/ml would have an 84% reduced chance to detect *KRAS* mutation compared with a respondent with CA19-9 levels >37 IU/ml. The regression coefficient

for CEA levels was not significant (B= −0.45, OR=0.64, p=0.293), indicating that CEA levels did not have a significant effect on the detection of *KRAS* mutation. The regression coefficient for metastasis was not significant (B= −0.48, OR=0.62, p=0.284), indicating that the presence of metastasis did not have a significant effect on *KRAS* mutation detection. Similarly, gender, tumor location, hemoglobin, and age did not show any significant effects on the detection of KRAS mutation.

Discussion

CRC initiation and progression are complex processes, involving several hallmark events that are well-known to be useful for determining disease prognosis. In this report, we attempted to systematically study well-known parameters that reflect the progression of CRC. We studied a cohort of 183 patients, from PNOC, and analyzed their CA19-9 and CEA levels and *KRAS* mutation status. Furthermore, these factors were analyzed to determine whether any significant associations existed among them, in addition to associations with tumor size, TNM stages, and distant metastases. We also studied the prognostic value of these three factors for predicting OS.

Abnormal levels of CA19-9 and CEA in Metastatic CRC (mCRC) have been reported in many studies, with varying proportions of patients showing elevated levels of CA19-9 and CEA [26,27]. However, our patient cohort exhibited a lower percentage of patients with abnormal CA19-9 levels than previous studies. CEA levels were high in slightly more than half of the patients in our cohort, which may be due to the low number of T4 patients in our cohort. Our comprehensive analysis of CEA and CA19-9 levels and *KRAS* mutation in CRC may be helpful for decision-making in mCRC patients. Earlier, a similar study examining these three markers suggested that high CEA and CA19-9 levels could act as predictors for the presence of *KRAS* mutation [28]. Our results further confirmed that abnormal CA19-9 levels were more likely to be observed in patients with mutated *KRAS*. Our observation of abnormally high CEA levels in patients with abnormal CA19-9 levels suggested that either of these two markers could be useful for analyzing *KRAS* status. Previously, *NRAS* mutation status has been associated with tumor size [29]. We report that the significant association between *KRAS* mutation and tumors >6 cm could also be indicative of advanced disease. In our study, tumors >6 cm showed abnormal levels of CEA. A high CEA level/tumor size ratio has been suggested to be a predictor of worse prognosis in CRC [30].

Abnormal CEA levels were observed in advanced tumor stages (T4, N2, M1) in our patient cohort but have not previously been demonstrated to be a significant factor [31]. Differences between our study and previous studies could be due to differences in the patient characteristics or ambiguity in the determination of TNM stages. In our cohort, pronounced differences were not visible in the T3 and N1 stages, in terms of abnormal CEA levels. However, CA19-9 and mutant *KRAS* were significantly different depending on lymph node status and the presence of distant metastases. The most significant difference was observed for abnormal CA19-9 levels in liver metastases. The liver is the most common metastases site associated with CRC and has been associated with elevated CA19-9 levels, in previous studies [32]. Although CA19-9 levels have been correlated with advanced pancreatic cancer, our study suggested that CA19-9 levels may also act as a significant indicator of pancreatic metastases. CA19-9 and CEA levels were significantly correlated with distant metastases to the lung and liver [33]. These results further supported our observations of abnormal CEA and CA19-9 levels in liver and lung metastases.

The prognostic value of CA19-9 levels in our patient cohort suggesting that CA19-9 may represent a promising biomarker, which should be verified in a larger patient cohort, in a prospective manner. Previous studies have shown the reduced survival of patients with elevated levels of CA19-9 [34], which agrees with our results. However, our results did not identify CEA levels or *KRAS* mutations as significant survival predictors. Differences between our study and other studies could be due to differences in the patient characteristics and the non-homogenous distribution of patients, in terms of age, gender, and tumor stages.

CRC is considered a disease of the elderly. However, many young patients are increasingly diagnosed with CRC [4,6]. Our observation of the significantly increased incidence of *KRAS* mutation in patients 45 years and older confirms the results reported by a recent comprehensive study that involved a large dataset of 13,336 CRC tumors, which showed that increased age, gender, microsatellite instability status, and tumor location were associated with KRAS mutation. Specifically, the Q61K mutation was identified among older, female patients. However, this study did not analyze the mutations associated with survival patterns [35].

The current comprehensive analysis of three important biomarkers associated with CRC provided evidence regarding the potential use of CA19-9 levels in the studied population. These details are useful for advancing the concept of precision medicine [36].

Take Home Massages

- CA19-9 may represent a strong prognostic biomarker.
- CEA and CA19-9 levels can be used to indicate *KRAS* mutation status and can be used as surrogate biomarkers.
- Abnormal CEA and CA19-9 levels are associated with advanced CRC.
- *KRAS* mutations are more prevalent among the older population.

Funding

This work was sponsored by King Abdullah International Medical Research Center (KAIMRC).

Acknowledgement

We thank King Abdullah International Medical Research Center (KAIMRC) for technical support.

References

1. ACS. Cancer Facts & Figures 2018. Atlanta, American Cancer Society, 2018.
2. Al-Shahrani ZSA-RAI, Al-Madouj AN, Hayder MS. Cancer incidence report Saudi Arabia 2014. Kingdom of Saudi Arabia Saudi Health Council, Saudi Cancer Registry, 2018.
3. Ministry of Health of Kingdom of Bahrain. Ten years cancer incidence among nationals of the GCC States 1998–2007, 2011.
4. Aziz MA, Allah-Bakhsh H. Colorectal cancer: A looming threat, opportunities, and challenges for the Saudi population and its healthcare system. Saudi J Gastroenterol. 2018;24(3):196-7.
5. Tan C, Du X. KRAS mutation testing in metastatic colorectal cancer.

World J Gastroenterol. 2012;18(37):5171-80.

6. Alhumaid A, Al-Yousef Z, Bakhsh HA, Al-Ghamdi S, Aziz MA. Emerging paradigms in the treatment of liver metastases in colorectal cancer. Crit Rev Oncol Hematol. 2018;132:39-50.
7. Aziz MA. Chapter 6. Colorectal cancer metastasis. In: Ahmad A, editor. Introduction to Cancer Metastasis. New York, Academic Press, 2017. p. 95-116.
8. Sartore-Bianchi A, Moroni M, Veronese S, Carnaghi C, Bajetta E, Luppi G, et al. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. J Clin Oncol. 2007;25(22):3238-45.
9. Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tse A, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol. 2005;23(9):1803-10.
10. Bokemeyer C, Cutsem EV, Rougier P, Ciardiello F, Heeger S, Schlichting M, et al. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: Pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer. 2012;48(10):1466-75.
11. Lin AY, Buckley NS, Lu AT, Kouzminova NB, Salpeter SR. Effect of KRAS mutational status in advanced colorectal cancer on the outcomes of anti-epidermal growth factor receptor monoclonal antibody therapy: A systematic review and meta-analysis. Clin Colorectal Cancer. 2011;10(1):63-9.
12. Imamura Y, Morikawa T, Liao X, Lochhead P, Kuchiba A, Yamauchi M, et al. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. Clin Cancer Res. 2012;18(17):4753-63.
13. Phipps AI, Buchanan DD, Makar KW, Win AK, Baron JA, Lindor NM, et al. KRAS-mutation status in relation to colorectal cancer survival: The joint impact of correlated tumour markers. Br J Cancer. 2013;108(8):1757-64.
14. Mattar R, Alves de Andrade CR, DiFavero GM, Gama-Rodrigues JJ, Laudanna AA. Preoperative serum levels of CA 72-4, CEA, CA 19-9, and alpha-fetoprotein in patients with gastric cancer. Rev Hosp Clin Fac Med Sao Paulo. 2002;57(3):89-92.
15. Carpelan-Holmström M, Louhimo J, Stenman UH, Alfthan H, Haglund C. CEA, CA 19-9 and CA 72-4 improve the diagnostic accuracy in gastrointestinal cancers. Anticancer Res. 2002;22(4):2311-6.
16. Qin XL, Wang ZR, Shi JS, Lu M, Wang L, He QR. Utility of serum CA19-9 in diagnosis of cholangiocarcinoma: In comparison with CEA. World J Gastroenterol. 2004;10(3):427-32.
17. Ni XG, Bai XF, Mao YL, Shao YF, Wu JX, Shan Y, et al. The clinical value of serum CEA, CA19-9, and CA242 in the diagnosis and prognosis of pancreatic cancer. Eur J Surg Oncol J. 2005;31(2):164-9.
18. Chen CC, Yang SH, Lin JK, Lin TC, Chen WS, Jiang JK, et al. Is it reasonable to add preoperative serum level of CEA and CA19-9 to staging for colorectal cancer? J Surg Res. 2005;124(2):169-74.
19. Przygotowanie DK. Składników testuradio immunologic znegoienzymo immunologic znegodla oznaczeń progesteronu, oczyszczanie frakcjonowanie przeciwciał: IgG, F(ab)'2 przeciw progesteron owi przeciw ferrytyn iedlapotrzeb diagnostyki klinicznej. Rozprawahabilitacyjna. Katowice; 1993. p. 234.
20. Park IJ, Choi GS, Jun SH. Prognostic value of serum tumor antigen CA19-9 after curative resection of colorectal cancer. Anticancer Res. 2009;29(10):4303-8.
21. Yang XQ, Chen C, Peng CW, Liu SP, Li Y. Carbohydrate antigen 242 highly consists with carbohydrate antigen 19-9 in diagnosis and prognosis of colorectal cancer: Study on 185 cases. Med Oncol. 2012;29(2):1030-6.
22. Morris-Stiff G, Teli M, Jardine N, Puntis MC. CA19-9 antigen levels can distinguish between benign and malignant pancreaticobiliary disease. Hepatobiliary Pancreat Dis Int. 2009;8(6):620-6.
23. Singh S, Tang SJ, Sreenarasimhaiah J, Lara LF, Siddiqui A. The clinical utility and limitations of serum Carbohydrate Antigen (CA19-9) as a diagnostic tool for pancreatic cancer and cholangiocarcinoma. Dig Dis Sci. 2011;56(8):2491-96.
24. Yue T, Maupin KA, Fallon B, Li L, Partyka K, Anderson MA, et al. Enhanced discrimination of malignant from benign pancreatic disease by measuring the CA 19-9 antigen on specific protein carriers. PloSOne. 2011;6(12):e29180.
25. Roche FH-L. Cobas® *KRAS* Mutation Test identifies mutations in codons. 2016;12:13.
26. Kim NH, Lee MY, Park JH, Park DI, Sohn CI, Choi K, et al. Serum CEA and CA 19-9 levels are associated with the presence and severity of colorectal neoplasia. Yonsei Med J. 2017;58(5):918-24.
27. Jia J, Zhang P, Gou M, Yang F, Qian N, Dai G. The role of serum CEA and CA19-9 in efficacy evaluations and progression-free survival predictions for patients treated with cetuximab combined with FOLFOX4 or FOLFIRI as a first-line treatment for advanced colorectal cancer. Dis Markers. 2019;2019:6812045.
28. Selcukbiricik F, Bilici A, Tural D, Erdamar S, Soyluk O, Buyukunal E, et al. Are high initial CEA and CA 19-9 levels associated with the presence of K-RAS mutation in patients with metastatic colorectal cancer? Tumour Biol. 2013;34:2233-39.
29. Guo TA, Wu YC, Tan C, Jin YT, Sheng WQ, Cai SJ, et al. Clinicopathologic features and prognostic value of *KRAS*, NRAS and BRAF mutations and DNA mismatch repair status: A single-center retrospective study of 1,834 Chinese patients with stage I-IV colorectal cancer. Int J Cancer. 2019;145(6):1625-34.
30. Cai D, Huang ZH, Yu HC, Wang XL, Bai LL, Tang GN, et al. Prognostic value of preoperative carcinoembryonic antigen/tumor size in rectal cancer. World J Gastroenterol. 2019;25(33):4945-58.
31. Topdagi O, Timuroglu A. Evaluation of the relationship between carcinoembryonic antigen and TNM stage in colorectal cancer. Eurasian J Med. 2018;50(2):96-8.
32. Dong H, Tang J, Li LH, Ge J, Chen X, Ding J, et al. Serum carbohydrate antigen 19-9 as an indicator of liver metastasis in colorectal carcinoma cases. Asian Pac J Cancer Prev. 2013;14(2):909-13.
33. Stiksma J, Grootendorst DC, van der Linden PW. CA 19-9 as a marker in addition to CEA to monitor colorectal cancer. Clin Colorectal Cancer. 2014;13(4):239-44.
34. Kouri M, Pyrhönen S, Kuusela P. Elevated CA19-9 as the most significant prognostic factor in advanced colorectal carcinoma. J Surg Oncol. 1992;49(2):78-85.
35. Serebriiskii IG, Connelly C, Frampton G, Newberg J, Cooke M, Miller V, et al. Comprehensive characterization of RAS mutations in colon and rectal cancers in old and young patients. Nat Commun. 2019;10:3722.
36. Aziz MA, Yousef Z, Saleh AM, Mohammad S, Al Knawy B. Towards personalized medicine of colorectal cancer. Crit Rev Oncol Hematol. 2017;118:70-8.