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

Subki AH1, Alghamdi TA2, Butt NS3, Alqazlan MS4, Alkahtani AM5, Aziz MA2 and Alsiary RA2*

1Department of Internal Medicine, King Faisal Specialist Hospital and Research Centre, Saudi Arabia
2Colorectal Cancer Research Program, Saudi Arabia/King Saud bin Abdulaziz University for Health Sciences, Saudi Arabia
3Department of Biostatistics, King Abdulaziz University, Saudi Arabia
4Department Anatomical Pathology, King Faisal Specialists Hospital, Saudi Arabia
5Royal Commission Yanbu Medical Center, Saudi Arabia

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 CA19-9 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 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

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].

Carciinoembryonic 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 <37IU/ml).
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
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=0.001). 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 distant metastasis showed abnormally high levels of CA19-9 and CEA. Among CRC patients with distant metastasis, 50% (n=19) presented with abnormal CA19-9 levels (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.
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 CEA19-9 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

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.

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

Note: $\chi^2(8)=19.32$, p=0.013, McFadden $R^2=0.10$
CA19-9 and CEA levels were significantly correlated with distant metastases. The liver is the most common metastases site associated with metastatic CRC (mCRC). Abnormal CA19-9 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 addition to associations with tumor size, TNM stages, and distant metastases. Abnormal CEA and CA19-9 levels are associated with advanced CRC. 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.

Take Home Messages
- CA19-9 may represent a strong prognostic biomarker.
- Abnormal CA19-9 levels can be used to indicate KRAS mutation status and can be used as surrogate biomarkers.
- 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.

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