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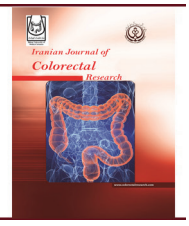

Cytokeratin Fragment 21.1 is a Useful Tumor Marker in Colonic Adenocarcinoma: A Cross-sectional Study

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Abstract

Background: Colorectal carcinoma is rising worldwide, representing a major cause of cancer-related mortality and morbidity. Carcinoembryonic antigen (CEA) is an established tumor marker for colorectal cancer, with uses in screening, pre-treatment staging, post-therapeutic monitoring, and recurrence detection. However, multiple factors affect CEA, including smoking and benign gastrointestinal diseases. Hence, there is a need to investigate alternative tumor markers like cytokeratin fragment 21.1 (CYFRA 21.1).
Methods: This study aimed to determine if the combination of CYFRA 21.1 and CEA is superior to CEA alone as a diagnostic marker in colonic cancer. In this cross-sectional study from June 2016 to December 2019, 69 consecutive patients with a histologically-confirmed diagnosis of colonic adenocarcinoma were studied. The serum levels of both tumor markers were analyzed before starting any definite treatment. The sensitivity and positive predictive values for both tumor markers were calculated. The correlation between tumor markers was tested using Pearson's correlation. The correlation between the TNM stage and tumor markers was tested using Spearman's Rho test.
Results: Forty-one patients had elevated CEA, while 33 patients had elevated CYFRA 21.1. CEA and CYFRA 21.1 mildly positively correlated with each other, with an R-value of 0.2598 (P=0.031). Spearman's correlation with the clinical stage of cancer was found to be 0.50834 for CEA (P<0.005) and 0.59828 for CYFRA 21.1 (P<0.005). The sensitivity of CEA was 59.42%, while that of CYFRA 21.1 was 47.83%. The combination of both had a sensitivity of 75.36%.
Conclusion: The combination of CYFRA 21.1 and CEA was more effective in picking up cases of colonic cancer than CEA alone. Both CYFRA 21.1 and CEA correlated well with the stage of the disease. Combining these biomarkers might has great potential to evolve as a diagnostic aid in colonic cancer.

Keywords: Adenocarcinoma, Colon cancer, Tumor markers, Carcinoembryonic antigen

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Introduction

Colorectal cancer (CRC) is inarguably a major global health burden. The tumor often progresses to metastasis and is sometimes incurable, often with a lengthy disease process. The natural history proceeds from an easily curable pre-malignant stage through an early, localized, mostly treatable malignant stage. The overall survival of CRC is quite good when compared with other cancers. However,

local recurrence is relatively common, even after radical curative surgery (1). The survival rates remain more favorable when the cancer is detected earlier: the stage-wise rates being 93%, 77%, 48%, and 7% at five years for diagnosis at stages I to IV, respectively (2). For this reason, early detection is crucial in improving these patients' disease-free periods and survival.

The fecal occult blood test (FOBT) remains one of the simplest methods for screening CRC. Results from several trials have reported a reduction in mortality to 15–33% while using FOBT-based screening (3). However, the potential benefits of FOBT are compromised by the limited sensitivity (13–50%) of detection in asymptomatic cohorts and the poor uptake (4, 5). Flexible sigmoidoscopy (FS) is the other test for first-line screening, complemented by colonoscopy when positive. FS offers better sensitivity over FOBT, picking up as much as 70–80% of advanced neoplasms of the colon and rectum (6). Though FS has proven to be efficacious for screening, it cannot be used to detect 40% of colonic tumors, which develop in the proximal segments (7, 8). The gold standard of screening is a colonoscopy, advocated only for high-risk groups in the United Kingdom but often employed for detecting sporadic cancers in the United States (9, 10). However, the compliance rates for these invasive tests remain on the lower side (11, 12).

Carcinoembryonic antigen (CEA) is the most common colorectal tumor marker. CEA is the one recommended by the American Society of Clinical Oncology and the National Academy of Clinical Biochemistry for assessing prognosis, monitoring response to treatment, and detecting metastases and recurrence (13, 14). CEA was first described by Gold and Freedman in 1965 when they identified it as an antigen that was detectable in both fetal colon and colonic adenocarcinoma but was absent from the healthy adult colon (15). Because of being present only in cancers and embryonic tissues, the protein was given the name CEA. Though the CEA is a cost-effective indicator of CRC, false positive elevation is frequently reported in smokers (16, 17). In addition to the above, several benign gastrointestinal diseases like ulcerative colitis, viral hepatitis, alcohol-related cirrhosis, and cryptogenic or biliary cirrhosis can potentially cause an increase in CEA levels (18).

Cytokeratin 19 is a kind of cytokeratin comprised of keratin and intermediate filaments of epithelial cells (19, 20). Circulating cytokeratin fragment 21.1 (CYFRA 21.1) is a biological tumor indicator reflecting fragments of cytokeratin 19. CYFRA 21.1 has already been proven to be a reliable biomarker in various malignancies, particularly that of the head, neck, and lungs (20, 21). The diagnostic performance of CYFRA 21.1 for CRC has been evaluated in some studies. However, its potential as a screening marker has not been previously assessed. Since CYFRA 21.1 is less vulnerable to factors like age, gender, and smoking history, CYFRA 21.1 may be better than CEA as a marker in the initial diagnosis and staging of CRC (22).

The search continues to find serum tumor markers other than or better than CEA for diagnosing CRC. An ideal biomarker would allow for easy diagnosis when the cancer is in its early stages, even before it starts its spread to other organs. It could ideally help clinicians to carry out patient stratification and to make optimal decisions about treatments. Furthermore, it can act as a predictor of overall outcomes and tumor recurrence. This concept formed the basis for this prospective single-center study, where we attempted to test the diagnostic efficacy of CYFRA 21.1 in combination with CEA.

The primary objective of this study was to find if a combination of CYFRA 21.1 and CEA is superior to CEA alone as a diagnostic marker in patients with CRC. The secondary objective was to find the correlation between CYFRA 21.1 and the cancer stage in these patients.

Patients and Methods

The current study had a cross-sectional design and was carried out for a period of three years, from 1st June 2016 to 31st December 2019, at the General Surgery and Oncology wards of Government Medical College Trivandrum, Kerala, India.

The inclusion criteria deemed eligible adult patients aged 12 years or older, with histology-confirmed adenocarcinoma of the colon or rectum, admitted to our wards for any definitive treatment. The exclusion criteria ruled out patients with previously diagnosed cancers of any site to avoid interference with the values of the tumor markers. Patients with any previous treatment for the current cancer were also excluded.

Approval from Institutional Review Committee and clearance from Human Ethics Committee (IEC No. 02/09/2016/MCT dated 26/03/2016) were obtained before commencing the study. Blood samples were collected before the start of definitive surgery or chemotherapy. The subjects were briefed about the study procedure in detail, and informed consent and signatures were obtained before the data and sample collection.

The sample size for the study was estimated using the recommended formula for sample size estimation in diagnostic test studies, wherein the sensitivity of the new test and established test were taken from reference studies (22). With an acceptable power of 80% and an alpha error of 5%, the sample size was calculated at 69, which was set as the study sample size. CEA was measured using a solid-phase, two-site chemiluminescent enzyme immunometric assay, while CYFRA 21.1 was measured with a commercially available enzyme-linked immunosorbent assay kit. The normal range

of CEA was taken as below 5 µg/l, with a greater cut-off of 7 µg/l among smokers. The normal range of CYFRA 21.1 was set as below 1.96 ng/ml. Histopathological confirmation of cancer was considered the gold standard reference.

Statistical Analysis

As part of the data collection, a data gathering checklist was created to record the subjects' clinical details. These included presenting features, clinical examination findings, and relevant investigation results. Both tumor markers' sensitivity and positive predictive value (PPV) were calculated. The correlation between the tumor markers was tested using Pearson's correlation test. The correlation between the TNM stage of colonic cancer and tumor markers was tested using Spearman's Rho test. Statistical analysis was carried out with the help of Microsoft Office Excel and EpiInfo software (CDC, Atlanta). Data are reported as arithmetic means±standard deviation and frequencies with the percentage in parentheses. Significance was considered at $P<0.05$.

Results

We studied 69 patients with colon cancer. The mean age was 60.33±10.99 years, with a maximum of 80 and a minimum of 38 years. There were 37 males (53.62%) and 32 females (46.38%). Eighteen patients had primary cancer in the rectum (26.09%), 18 in the sigmoid colon (26.09%), 9 in the cecum (13.04%), 7 in the transverse colon (10.14%), 6 in the ascending colon (8.69%), 5 in the splenic flexure (7.25%), 4 in the descending colon (5.79%), and 2 in the hepatic flexure (2.89%).

The mean values of CEA and CYFRA 21.1 were 27.71±62.85 µg/l and 10.45±20.08 ng/ml, respectively. Among the 69 patients, 41 had elevated CEA, while 33 had elevated CYFRA 21.1. Fifty-two patients were positive for either marker (Figure 1). The sensitivity of CEA was calculated at 59.42% (CI 46.92% to 71.09%), and that of CYFRA 21.1 was 47.83% (CI 35.65% to 60.20%). CEA had a PPV of 3.03%, while CYFRA 21.1 had a PPV of 2.46%. The combination of CEA and CYFRA 21.1 had a sensitivity of 75.36% (CI 63.51% to 84.95%), with a PPV of 3.82% (Table 1).

When Pearson's correlation was tested, the correlation between CEA and CYFRA 21.1 was calculated at 0.2598, indicating a mild positive correlation ($P=0.031$). Pearson's correlation between age and CEA was negative, with an R-value of -0.2613, while that for age and CYFRA 21.1 was also negative, with an R-value of -0.261. Sixteen patients presented in stage 1 of cancer (23.19%), 28 in stage 2 (40.58%), 19 in stage 3 (27.54%), and 6 in stage 4 (8.69%). CYFRA 21.1 correlated better with the disease stage than CEA (Figure 2). Spearman's correlation was tested between CEA and cancer stage; the rho value was 0.50834, which was significant ($P<0.005$). For CYFRA 21.1, the rho value was 0.59828, which was also significant ($P<0.005$). ANOVA revealed a significant relationship between the cancer stage and each marker, with an f-ratio value of 27.17364 ($P<0.00001$) for CYFRA 21.1 and 3.01865 for CEA ($P=0.036038$). The two-tailed test of the difference between the two markers was significant at a P-value of 0.0316 for a t-value of 2.1722.

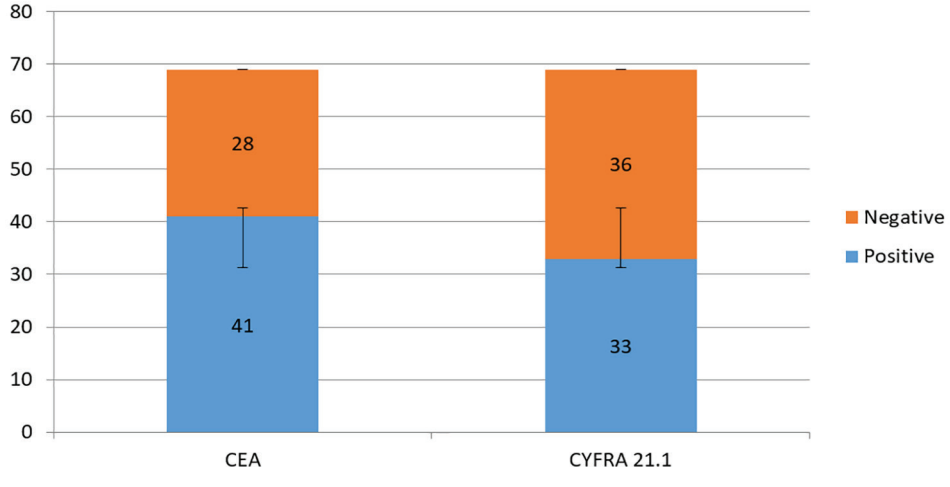

Figure 1: Distribution of positivity of the tumor markers.

Table 1: Performance characteristics* of CEA and CYFRA 21.1

	Sensitivity	Positive predictive value
CEA	59.42%	3.03%
CYFRA 21.1	47.83%	2.46%
CEA+CYFRA 21.1	75.36%	3.82%

*Specificity and negative predictive value could not be determined considering the study design. CEA: Carcinoembryonic antigen CYFRA 21.1 : cytokeratin fragment 21.1

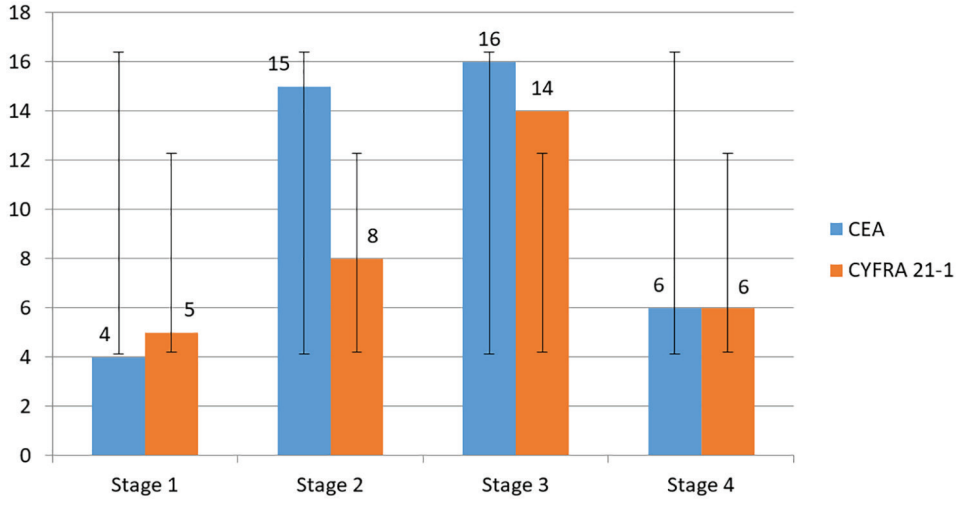

Figure 2: Stagewise distribution of the tumor markers. Spearman correlation for CEA: 0.50834, P<0.005; Spearman correlation for CYFRA 21.1: 0.59828, P<0.005; Significant difference between markers in the two groups: t-value=2.1722, P=0.0316

Discussion

In colorectal cancer, CEA has been in application universally, right from the start of pre-treatment staging to assessment of recurrence and response to chemotherapy regimens. CEA is a practical tool for suspecting metastasis or relapse and a predictive marker of worse prognosis when high preoperative levels do not reduce to normal ranges after resection (15). However, the high false-positive results and the lower sensitivity of CEA in the pre-treatment evaluation setting reflect that stand-alone CEA might be an unsuitable agent for population screening (23).

Most of the available literature supports our study findings. In a study, at a threshold of 5 ng/ml, the sensitivity of CEA for detecting CRC up to 1 and 4 years before the clinical presentation was 25% and 13%, respectively, at a specificity of 95% (24). At a threshold of 2.5 ng/ml for CEA, sensitivity for CEA and CYFRA 21.1 were 57.5% and 38.4%, respectively, with a specificity of 81% and 83.5%. CYFRA 21.1 and CA 125 were found to have no utility as screening markers and also did not add to the performance of CEA when employed in combination. Some studies suggest that only some subsets of CRC produce an elevation in serum CEA levels, which is specific to the malignant phenotype (25). Rising levels of CEA have been detected to be much more frequent in late-stage tumors (26). However, CEA levels often do not correlate with tumor grade, as suggested by previous studies (27).

Serum CEA has also been found to have very limited sensitivity for screening when used in asymptomatic people. In a study on 46 preclinical cases (29 were of early stage/17 were of an advanced stage), testing for CEA provided a lead time of up to two years in only 30% of future CRCs when a cut-off threshold was used that correctly identified 99% of the controls (28). In another research, elevated CEA levels provided a lead time of up to 7 months only in 19% of the 32 preclinical cases studied (17 were of early stage/15 were of an advanced stage) (29). However, both these studies involved the analysis of single cross-sectional design sample units and were limited to a maximum lead time of two years.

Though CEA is superior to the guaiac FOBT, it does appear to be inferior to Cologuard®. Cologuard is a fecal test that combines hemoglobin protein, seven KRAS gene point mutations, NDRG4 and BMP3 gene promoter hypermethylation, and b-actin DNA as a normalization marker (3, 4). CEA is also inferior to Epi proColon® (plasma SEPT9 DNA methylation), which has been evaluated in large prospective trials, and also the fecal immunochemical test, which is a more precise version of the FOBT for detecting hemoglobin (30-33). It is also important to note that the administration of 5-fluorouracil-based therapy can cause significant transient increases in CEA levels even if there is no disease progression. In research by Moertel et al., among the 99 subjects who developed liver toxicity while on chemotherapy, 19 patients had a false-positive increase in CEA levels. Their CEA values ranged from 5.1 to as high as 34 mg/l and subsequently returned to normal after cessation of chemotherapy (34).

Gawel et al. tested endoscopy trial specimens for a panel of biomarkers including CYFRA 21.1, alpha-fetoprotein, carbohydrate antigen (CA) 19-9, and CEA, and were able to develop an accurate algorithm for predicting high-risk adenomas as well as colorectal cancers with (35). In the study by Lim et al., CYFRA 21.1 showed significant diagnostic performance as well as great step-wise comparative potential when differentiating patients with colonic adenomas from benign controls (36).

There are also some studies that reveal findings different from ours. One study found that CYFRA 21.1 (cut-off≥1.13 ng/ml) had a sensitivity of 47% when compared with 37% for CEA (cut-off≥3.05 ng/ml) and 32.6% for CA 19.9 (cut-off≥23.1 ng/ml) when used in the initial staging work-up of primary CRC (26). When different cut-off values were used, CYFRA 21.1 showed a higher sensitivity for pre-treatment detection than CEA and CA 19.9

in colorectal adenocarcinoma in this study. The authors also noted a mildly significant correlative relationship between Dukes' stages and all three tumor markers (P<0.01). The areas under the receiver operating characteristic curves (AUC) for CYFRA 21.1, CEA, and CA 19-9 were 0.81±0.03, 0.74±0.03, and 0.62±0.04, respectively, when used for discriminating CRC from benign colorectal conditions. In addition to the above, CYFRA 21.1 was the most sensitive tumor marker among the three for detecting recurrent CRC at all cut-off values.

Xu et al. demonstrated that cytokeratin 19 mRNA was detectable in 41.9% of patients with CRC and in 3.3 % of controls (37). This sensitivity was evidently higher than the CEA mRNA detection rate (35.8% of CRC patients and 3.3% of controls). Holdenrieder et al. also showed that CYFRA 21.1 levels were predominantly higher in patients with CRC when compared with healthy controls (P<0.001) and also other benign gastrointestinal diseases (P=0.01), and even showed significant stage dependency (P=0.01) (38). Dressen et al. evaluated the diagnostic performance of a multiplex immunoassay panel that included CEA, CA 19-9, and CYFRA 21.1, revealing that a combination of CA 19-9 and CEA had the best diagnostic performance with a higher AUC (39). In their study, CEA showed the best performance as a single marker. In a study by Thomas et al., CYFRA 21.1 and CA 125 had no utility as screening markers and did not enhance the performance of CEA when used in combination (24).

The present study is not without its own set of limitations. Since we included only confirmed cases, we could not find the AUC for each tumor marker. Due to the study design, no follow-up was done to analyze prognosis or survival. Last, the sample size was comparatively small, which, along with the study's single-center setting, could mitigate the validity and generalizability of the study results.

Conclusion

A combination of CEA and CYFRA 21.1 can pick up more colon cancer cases than either of them alone. The poor sensitivity of CEA and CYFRA 21.1 make either of them useless as stand-alone screening tools for colon cancer. Both CEA and CYFRA 21.1 correlate well with the stage of cancer at presentation. In conclusion, CYFRA 21.1 holds great promise as a tumor marker in combination with CEA in colon cancer. The findings of this study demand the need for further large-scale trials to assess the potential of CYFRA 21.1. The results from our study could act as the groundwork for building and subsequently assessing longitudinal algorithms for CRC screening. Also, there is a potential for combining other promising new biomarkers with CEA to add to its performance.

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Authors' Contribution

Study concept and design: Dr Meer Chisthi.M, Dr Krishnakumar KG; Acquisition of data: Dr Meer Chisthi.M, Dr Krishnakumar KG; Analysis and interpretation of data: Dr Meer Chisthi.M, Dr Viswanathan KV; Drafting of the manuscript: Dr Meer Chisthi.M, Dr Viswanathan KV; Critical revision of the manuscript for important intellectual content: Dr Meer Chisthi.M, Dr Viswanathan KV; Statistical analysis: Dr Meer Chisthi.M, Dr Krishnakumar KG; Administrative, technical, and material support: Dr Krishnakumar KG, Dr Viswanathan KV; Study supervision: Dr Krishnakumar KG, Dr Viswanathan KV. All authors have read and approved the final version. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest: None declared.

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