

Assessment of long noncoding RNA CCAT1 using real time-polymerase chain reaction in colorectal cancer patients

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Abstract

Colorectal cancer (CRC) is linked to high mortality, mainly when discovered in its advanced stages. Several studies have pointed to the role of epigenetic factors in CRC and other cancers. Long non-coding RNAs (lncRNAs) are involved in the initiation, progression, metastasis, and modulation of the response to chemotherapeutic modalities of CRC as vital contributors to epigenetic mechanisms. Colon cancer-associated transcript-1 (CCAT1) is one of the lncRNAs that have been dysregulated in serum samples, providing a non-invasive route for diagnosing CRC patients. This study aimed to determine the role of CCAT1 expression as diagnostic and prognostic markers. We tested the associations of CCAT1 expression with serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9). The study included three groups: 41 patients with colorectal cancer, 39 patients with precancerous benign colorectal diseases, and 20 normal control individuals. CEA and CA 19-9 were measured by an immunoassay automated system. The expression level of CCAT1 was assessed by a real-time polymerase chain reaction. There was a statistically significant elevation of serum CEA levels in patients with CRC compared to patients with precancerous benign colorectal diseases. Furthermore, there was no statistically significant difference in serum CA 19-9 levels between all groups ($p = 0.102$). Interestingly, CCAT1 expression was significantly upregulated in the blood of CRC patients compared to the precancerous benign colorectal diseases group ($p = 0.009$) and the control group ($p < 0.001$). Also, expression of CCAT1 was significantly elevated in patients with precancerous benign colorectal diseases compared to the control group ($p = 0.004$). In conclusion, measuring the expression level of CCAT1 is more advised than assessment of CEA and CA 19-9 for the early diagnosis and prognosis of colorectal cancer.

Keywords: Colorectal cancer, carcinoembryonic antigen, CA 19-9, CCAT1.

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Introduction

The fourth most prevalent cancer diagnosed worldwide is colorectal cancer (CRC), which is also the third most deadly malignancy. An estimated two million new cases and one million deaths occurred in 2018.¹ In Egypt, CRC was estimated at 6.5 % of all malignant tumors. The disease is also widespread in Egypt, revealing 14.0 % of all colonoscopies. In addition, it affects both men and women (4.2% and 3.8%, respectively) and it was the 3rd most reported cancer in males and the 2nd most common cancer in females.² CRC survival rates are highly dependent upon the disease stage at diagnosis: at the localized stage, it is 90%; 70% at regional disease, and 10% for patients diagnosed with distant metastasis.³

Inflammatory bowel disease (IBD), considered a risk factor for CRC, represents 1–2% of all CRC cases. Longitudinal investigations have shown that the severity of colonic inflammation, the length of bowel involvement, and the presence of primary sclerosing cholangitis are all indicators of the development of CRC.^{4–6}

It was suggested that CRC patients could be diagnosed using non-invasive methods.⁷ In CRC patients who received surgical resection and adjuvant treatment, carcinoembryonic antigen (CEA) is a potent predictive indicator. A poor prognosis is related to an increased CEA level of >5 g/L at the time of the diagnosis of a new CRC case.⁸ However, returning increased CEA levels to normal levels following surgery is not related to a negative prognosis. The predominant tumor marker predictive of CRC is still CEA, and carbohydrate antigen 19-9 (CA 19-9) which are not efficient in the diagnosis of CRC. Moreover, the staging, prognosticating, and managing of metastatic CRC were done by the CA 19-9 assay.⁹ Particularly in individuals with metastatic CRC, CA 19-9 has demonstrated the ability to serve as a treatment guide. Additionally, CA 19-9 might identify metastatic CRC patients with potentially worse survival results for more intensive care.⁹

One of the most common malignancies that is influenced by epigenetic factors is CRC. RNA molecules located in the non-coding regions of

the genome are known as non-coding RNAs (ncRNAs).¹⁰ There are several motivating factors, including long ncRNAs (lncRNAs) and DNA methylations. During the phases of cancer development, lncRNAs often perform complicated molecular functions that take place at several levels, including the transcriptional, post-transcriptional, and epigenetic levels.¹¹

Several studies particularly those conducted in the last decade indicated that epigenetic variables have a role in colorectal cancer.^{12–14} In the onset, development, and metastasis of CRC, lncRNAs play a significant role in the epigenetic pathways. The colon cancer-associated transcript-1 (CCAT1) gene produces lncRNA which was found to be significantly expressed in a variety of malignancies, including hepatocellular, gallbladder, and CRC adenomas and adenocarcinomas at all stages.¹⁵

CCAT1 plays a crucial role in several biological processes, including proliferation, invasion, migration, drug resistance, and overall survival. It was consistently raised for a variety of cancer types.¹⁶ Dereglulation of CCAT1 affects carcinogenesis as well as clinical traits such as tumor size, regional lymph nodes, and distant metastases (TNM) stage, invasion, and patient survival¹⁶. Consequently, this study aimed to determine the role of CCAT1 expression as diagnostic and prognostic markers. In particular, we tested the associations of CCAT1 expression with serum CEA and CA 19-9.

Subjects and Methods

This study was carried out in the laboratory of the Department of Clinical Pathology, Assiut University Hospital, Faculty of Medicine, Assiut University during the period December 2020 and June 2022.

The study included 41 newly diagnosed CRC patients (16 females and 25 males); their ages ranged from 19 to 86 years old. Diagnosis and staging were based on colonoscopy findings, abdominal radiography, pathological findings, and clinical decisions. The staging was based on TNM according to the American Society of Clinical Oncology staging system¹⁷.

The study also included 39 patients with precancerous benign colorectal diseases, of whom 8 had ulcerative colitis, 10 had Crohn's disease, and 21 had non-specific colitis. Their ages ranged from 20 to 75 years. All patients (CRC and precancerous) were recruited from the Department of General Surgery, Assiut University Hospital, Assiut University, and the Department of Surgical Oncology, South Egypt Cancer Institute, Assiut University. In addition, 20 normal individuals (16 females and 4 males) were selected as a control group. Their ages were matched to patient groups. The practical work was carried out at the Department of Clinical Pathology, Assiut University Hospital.

Patients with CRC who had received chemotherapy, radiotherapy, or surgical treatment and those that had a history of non-precancerous benign colorectal diseases (irritable bowel syndrome, appendicitis, diverticulitis, paralytic ileus, and intussusception) or patients with a history of benign or malignant tumors in other organs were excluded from this study.

A blood sample (3 ml) was collected from each study subject and used for the separation of serum. Sera were used for measurement of CEA and CA 19-9. CEA and CA 19-9 were determined using an automated immunoassay system (CEA: Cat # 00937450 and CA19-9: Cat #: 10491379, ADVIA Centaur Auto-Analyzer, Siemens Healthineers, USA), according to the manufacturer's instructions.

In addition, a blood sample (2 ml) was collected from each study subject into an EDTA-coated tube for detection of CCAT1 in the plasma using the polymerase chain reaction (PCR). Purification of total RNA from human whole blood was performed using commercial kits (Cat # 52304, QIAamp RNA Blood Mini Kit, QIAGEN, Germany), according to the manufacturer's instructions.

Reverse transcription was used to prepare cDNA and performed with commercial kits (Cat # K1622, Revert Aid First Strand cDNA Synthesis Kit, Thermo-Fisher Scientific, USA), according to the manufacturer's instructions. Then detection of CCAT1 was performed by a quantitative real-

time PCR (qRT-PCR) assay using commercial kits (Cat# K0251, Thermo-Fisher Scientific, USA), according to the manufacturer's instructions. The kits included qPCR Master Mix (Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) and a primer set (CCAT1 (Cat#330701 LPH15969A) and (GAPDH) was used as an internal control (Cat#330701 LPH31725A). The cDNA prepared in the reverse transcription reaction served as the template for real-time PCR analysis.

The qRT-PCR was performed using a real-time PCR system (7500 fast real-time PCR, Applied Biosystems, USA). The qRT-PCR was programmed according to the following conditions: incubation at 95°C for 10 min as a preliminary activation step for HotStarTaq DNA polymerase, followed by 40 amplification cycles, each of a DNA denaturation at 94°C for 15 s, an annealing at 60°C for 60 s, and an extension for 40 s. Fluorescence measurements were performed at each cycle.

Specific amplification of lncRNA was ensured through the analysis of the melting curves. The cycle threshold (Ct) value was defined as the cycle number at which there is the first detectable increase in fluorescence signals above a defined threshold. Ct values were automatically calculated.

2- $\Delta\Delta C_t$ method was used to calculate relative expression of lncRNAs.¹⁸

The results were expressed as Fold Change (FC). Normal value is assumed to equal 1.

$$\Delta C_t \text{ Sample} = C_t_{\text{CCAT1}} - C_t_{\text{GAPDH}}$$

$$\Delta C_t \text{ Control}_{\text{Mean}} = C_t_{\text{CCAT1}} - C_t_{\text{GAPDH}}$$

$$\Delta\Delta C_t \text{ Sample} = \Delta C_t \text{ Sample} - \Delta C_t \text{ Control}_{\text{Mean}}$$

$$\text{Relative quantitation (Fold Change) of sample} = 2^{-\Delta\Delta C_t}$$

$$\text{Relative quantitation (Fold Change) of control Mean} = 1$$

Statistical Methods

All statistical calculations were done using the statistical package for social science (SPSS, SPSS Inc., Chicago, IL, USA), version 26. Data were statistically described as mean $\pm$ standard deviation ($\pm$ SD), median, and range when not

normally distributed, frequencies (number of cases), and relative frequencies (percentages) when appropriate. Quantitative variables were compared using the Mann-Whitney U test for dichotomous non-normally distributed data. One-way ANOVA or Kruskal-Wallis tests were applied to compare three quantitative variables. To compare categorical data, the Chi square (χ^2) test was performed. Instead, the exact test was used when the expected frequency was less than 5. The correlation between different variables was done using the Pearson correlation test. The Receiver Operating Characteristic (ROC) curve analysis was used to find out the best cut-off values to validate the detection of CRC. A *p*-value was always two-tailed and set the significance at <0.05 level.

Results

The mean rank levels of CEA and CA 19-9 are reported in Table 1. There was a statistically significant elevation of serum CEA levels in patients with CRC compared to patients with precancerous benign colorectal diseases ($p = 0.035$). However, no difference was found between serum CEA levels in patients in the CRC group compared to the control group ($p = 0.101$), and also when precancerous benign colorectal diseases group compared to the control group ($p = 1$). Serum CA 19-9 levels showed no difference between all study groups ($p = 0.102$).

Table 1. Comparison of carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) levels in the studied groups.

Tumor markers	CRC cases (n=41)	Precancerous (n=39)	Controls (n=20)	<i>p</i> value
CA 19-9				
Mean $\pm$ SD	36.74 $\pm$ 78.04	10.87 $\pm$ 8.74	8.49 $\pm$ 9.78	NS
Median (range)	8.6 (1.2 – 431.3)	9.1 (1.2 – 40.9)	4.3 (1.2 – 37.0)	
CEA				$P^1 < 0.001$
Mean $\pm$ SD	9.06 $\pm$ 22.15	1.01 $\pm$ 0.69	1.02 $\pm$ 0.76	$P^2 = 0.035$
Median (range)	2.1 (0.5 – 100)	0.7 (0.5 – 3.3)	0.8 (0.5 – 3.4)	$P^3 = NS$
				$P^4 = NS$

CA 19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen. Quantitative data are presented as Mean $\pm$ SD and median (range). $p > 0.05$ is not significant (NS). The Kruskal-Wallis test was used for comparing continuous data with post-hoc analysis. P^1 : Comparison among all groups; P^2 : Comparison between CRC and precancerous cases.

P^3 : Comparison between CRC and controls, P^4 : Comparison between precancerous cases and controls.

Plasma CCAT1 expression in the studied groups is reported in Table 2. Plasma CCAT1 expression showed statistically significant elevation in patients in the CRC group compared to both the precancerous benign colorectal diseases group

($p = 0.009$) and the control group ($p < 0.001$). Also, patients with precancerous benign colorectal diseases had a statistically significant elevation of plasma CCAT1 expression in comparison to the control group ($p = 0.004$).

Table 2. Comparison of the colon cancer associated transcript-1 (CCAT1) expression between the studied groups.

	CRC cases (n=41)	Precancerous (n=39)	Controls (n=20)	<i>p</i> value
CCAT1 (FC)				$p^1 < 0.001$
Mean $\pm$ SD	7.01 $\pm$ 5.80	4.37 $\pm$ 1.76	0.81 $\pm$ 0.45	$p^2 = 0.009$
Median (range)	4.89 (1.06 – 20.25)	4.59 (1.26 – 7.06)	0.85 (0.15 – 1.66)	$p^3 < 0.001$
				$p^4 = 0.004$

CCAT1: colon cancer associated transcript 1; Quantitative data are presented as Mean $\pm$ SD and median (range). Significance is defined by $p < 0.05$. The Kruskal-Wallis test was used for comparing continuous data with post-hoc analysis.

P^1 : Comparison among all groups; P^2 : Comparison between CRC and precancerous cases

P^3 : Comparison between CRC and controls; P^4 : Comparison between precancerous cases and controls.

Expression of plasma CCAT1 between CRC stages (staging was based on TNM) is shown in Table 3. Advanced tumor stages (III + IV) of CRC

showed a statistically significant elevation of plasma CCAT1 expression in comparison to early tumor stages (I + II) ($p < 0.001$).

Table 3. Relative quantitation of colon cancer associated transcript-1 (CCAT1) expression in different stages of the 41 colorectal cancer patients.

Tumor stage	N	Mean $\pm$ SD	Median (range) fold change	p value
Early stage (I + II)	22	3.57 ± 1.93	2.96 (1.06 – 7.93)	<0.001
Advanced stage (III + IV)	19	10.99 ± 6.27	11.07 (2.22 – 20.25)	

CCAT1: colon cancer associated transcript 1; Quantitative data are presented as Mean $\pm$ SD and median (range). Significance is defined by $p < 0.05$. An ANOVA test was used for comparing continuous data.

Performance of CEA, CA 19-9 levels and plasma CCAT1 expression for diagnosis of cancer colon

A ROC curve analysis was done to assess the diagnostic performance of serum CEA, CA 19-9, and plasma CCAT1 expression in cancer colon and in controls (in discriminating malignant from apparently healthy controls included in our study). By plotting the ROC curve (Figure 1), the diagnostic efficacy was determined using the calculated cutoff point of 1.2 ng/ml, 5.24 U/ml, and 1.2-fold change for CEA, CA 19-9, and CCAT1, respectively. The area under the curve (AUC) was calculated as 0.757, 0.665, and 0.980, respectively.

Considering these cutoff points, the sensitivity, specificity, and accuracy of CEA for diagnosis of colorectal cancer was 63.4%, 80.0% and 68.9%, respectively, with a positive predictive value of 76.7% and a negative predictive value of 51.6%. The diagnostic performance of CA 19-9 for diagnosis of CRC was 70.5% accuracy, 73.2% sensitivity, and 65.0% specificity, with a positive predictive value of 81.1% and a negative predictive value of 54.2%. The diagnostic performance of CCAT1 for diagnosis of CRC was 88.5% accuracy, 92.7% sensitivity, and 80.0% specificity, with a positive predictive value of 90.5% and a negative predictive value of 84.2%.

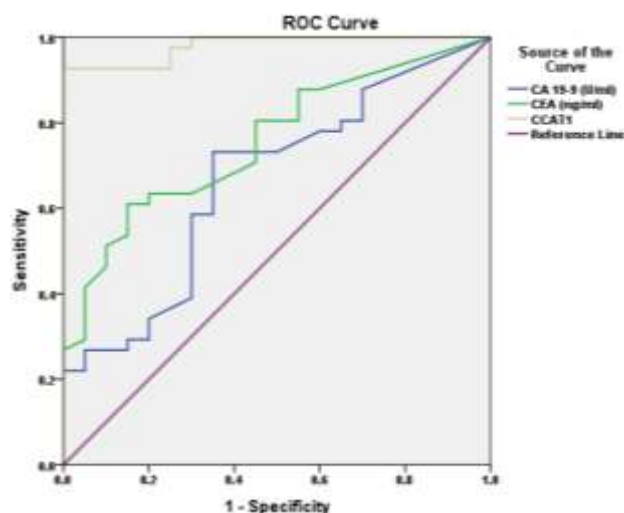


Figure 1. Receiver Operating Characteristic (ROC) curve analysis for detection of colorectal cancer (CRC). Carbohydrate antigen 19-9 (CA 19-9) (blue), carcinoembryonic antigen (CEA) (green), colon cancer associated transcript 1 (CCAT1) (brown), and Reference Line (purple). Area under the curve for CA 19-9 (U/ml) = 0.665 (0.52 to 0.81), $p = 0.038$, area under the curve for CEA (ng/ml) = 0.757 (0.64 to 0.88), $p = 0.001$, and area under the curve for CCAT1 = 0.980 (0.95 to 1.0), $p < 0.001$.

The performance of CEA, CA 19-9 level and plasma CCAT1 expression for diagnosis of precancerous disease detection

A ROC curve analysis was done to assess the diagnostic performance of serum CEA, CA 19-9, and plasma CCAT1 expression in precancerous diseases and controls (in discriminating precancerous diseases from controls included in our study). By plotting the ROC curve, the diagnostic efficacy was determined using the calculated cutoff point of 1 ng/ml, 5.34 U/ml, and 1.37-fold change for CEA, CA 19-9, and CCAT1, respectively. And the AUC was calculated as 0.506, 0.626, and 0.990, respectively.

Considering these cutoff points, CEA had 49.2% accuracy, 38% sensitivity, and 70.0% specificity, with a positive predictive value of 71.4% and a negative predictive value of 36.8%.

The diagnostic performance of CA 19-9 for diagnosis of CRC was 67.8% accuracy, 69.2% sensitivity, and 65.0% specificity, with a positive predictive value of 79.4% and a negative predictive value of 52.0%. The diagnostic performance of CCAT1 for diagnosis of CRC was 94.9% accuracy, 97.4% sensitivity and 90.0% specificity with positive predictive value 95.0% and negative predictive value 94.7%.

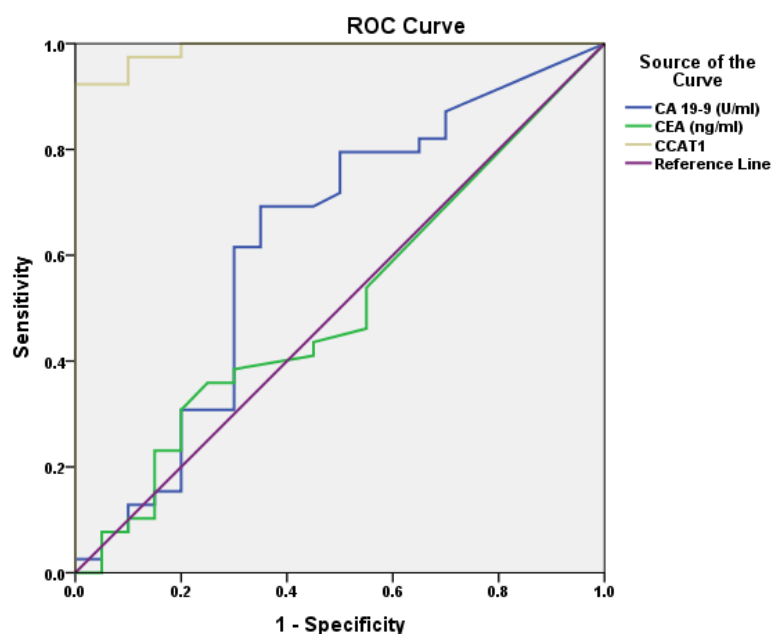


Figure 2. Receiver Operating Characteristic (ROC) curve analysis for detection of precancerous lesions: Carbohydrate antigen 19-9 (CA 19-9) (blue), carcinoembryonic antigen (CEA) (green), colon cancer associated transcript 1 (CCAT1) (brown), and Reference Line (purple). The area under the curve for CA 19-9 (U/ml) = 0.626 (0.47 to 0.79), $p = 0.115$; area under the curve for CEA (ng/ml) = 0.506 (0.35 to 0.66), $p = 0.943$; and area under the curve for CCAT1 = 0.990 (0.97 to 1.0), $p < 0.001$.

Discussion

CRC can be easily curable if diagnosed early. Despite great advances in cancer screening in recent years, the prognosis of colorectal cancer remains poor. Colonoscopy is the standard screening method for early detection of

colorectal cancer.^{19, 20} In addition, other CRC screening tests, such as sigmoidoscopy, fecal occult blood test, tumor markers, fecal immunochemical test, and radiology, have lower sensitivity and specificity or are more costly.²⁰

In this work, we tried to evaluate CEA, CA 19-9, and CCAT1 as non-invasive markers for diagnosis and prognosis of CRC patients and precancerous benign colorectal diseases. Our study revealed that serum CEA levels showed a statistically significant elevation in patients with CRC when compared to patients with precancerous benign colorectal diseases ($p = 0.035$). However, no difference was found between serum CEA levels in CRC patients compared to the control group ($p = 0.101$) and in the precancerous benign colorectal diseases group compared to the control group ($p = 1$). Serum CA 19-9 although showed a higher level in CRC patients when compared with the benign colorectal diseases group and the control group, and also when compared with the benign colorectal diseases group and the control group, but the difference did not reach statistical significance ($p = 0.102$).

The CEA is predicting the prognosis of CRC patients with high preoperative CEA levels and may aid in the development of postoperative treatment regimens for CRC patients.²¹ Many previous studies showed that the serum level of CEA was increased in many cancers and non-cancer diseases, such as ulcerative colitis and Crohn's disease.^{22, 23} However, the increased serum CEA level is not useful for detection of early disease or for diagnosing malignant growth. The high CEA level is in the cancer group, lower in the IBD group, and lowest in the colonic non-diseased margins.^{22, 23}

As Vukobrat-Bijedic et al., 2013, illuminated CEA and CA 19-9 have little significance in the early diagnosis of CRC because of their deficiency of sensitivity and specificity.²⁴ In 2020, Tümay & Guner, found a weaker and poorer prognostic worth of high CA 19-9 levels when used alone, suggesting the combined use of CEA and CA 19-9 markers in prognostic assessment and risk-adapted follow-up observation in CRC cases.²⁵ Similarly, Lakemeyer et al., 2021, did not recommend using both CEA and CA 19-9 in the screening program. However, before performing primary surgery and considering more aggressive treatment for patients with advanced CRC, they recommend measuring the tumor markers CEA and CA 19-9 for prognostic purposes.²⁶ The study by Subki et

al., 2021, provided new visions into the vital roles played by CA 19-9 and CEA during the progression of CRC and proposed that they may serve as suitable biomarkers in the management of CRC. Since it was found that the levels of CA 19-9 increased with increasing tumor stage.²⁷

Our study exhibited significantly higher expression levels (relative quantitation level) of CCAT1 in CRC patients compared to the precancerous benign colorectal diseases group ($p = 0.009$) and the control group ($p < 0.001$). Furthermore, CCAT1 expression levels showed a statistically significant elevation in patients with precancerous benign colorectal diseases when compared to the control group ($p = 0.004$). The degree of CCAT1 expression in plasma was also observed to be higher in CRC cases than in apparently healthy people. This opens the opportunity of developing liquid biopsy tests based on the detection of CCAT1 for a slightly invasive screening or diagnosis of CRC.²⁸

Numerous studies stated the altered expression of certain lncRNAs, including CCAT1, already in precancerous adenomas.^{29–31} Our results agreed with those of Ghafari et al., 2022, who reported that the expression levels of CCAT1 were dramatically increased, with 4.54 times more elevated levels in the blood of CRC cases contrasted to the levels seen in the controls.¹⁵ Alaiyan et al., 2013, proposed that the high expression of CCAT1 in both malignant and benign CRC, as compared to normal controls, makes it a viable diagnostic for early CRC detection.³²

Nissan et al., 2012, in their comprehensive RT-qPCR study, were the first to demonstrate the gigantic (often more than 100-fold) upregulation of CCAT1 in CRC and premalignant adenoma tissue samples contrasted to the normal colonic mucosa. On the other hand, for non-invasive diagnosis, CCAT1 is similarly overexpressed in 40% of peripheral blood samples taken from CRC cases but not from apparently healthy people.³³

The absence of a specific highest-quality level system for the diagnosis of IBD and the dependence of the present diagnostic approaches on invasive biopsy procedures require the improvement of harmless screening

methods. The observed deviant regulation of miRNAs/ lncRNAs in plasma samples of IBD patients has been a hopeful finding in such a manner.³⁴ Precancerous benign colorectal diseases CCAT1 elevation is attributed to CCAT1 promoting inflammatory response and cell migration in human intestinal epithelial cells.³⁴

Although the association between inflammation and cancer initiation is well recognized, whether CCAT1 is involved in inflammation and promotes IBD, malignancy stays unsure.³⁵ Ma et al., 2019, noticed a critical increase in CCAT1 expression in inflamed colonic tissues and primary colon adenocarcinoma. They also clarified inflammatory bowel disease, particularly ulcerative colitis, has been supposed to be a precancerous illness for CRC. Mechanistically, chronic inflammation of the colon epithelia has been shown to lead to CRC initiation.³⁵

In our study, CCAT1 levels showed significant elevation with the advanced tumor stages of CRC ($p < 0.001$) as Shang et al., 2020, showed that CCAT1 expression level was positively related to the advanced stage, lymph node metastasis, distant metastasis, or vascular invasion ($p < 0.05$) of CRC.³⁶ Also, Zhang et al., 2022, found that CCAT1 was upregulated in the early stages of colorectal carcinogenesis and related to the TNM stage.³⁷

lncRNAs have been studied in tumor initiation and progression, in light of their impacts on cellular and molecular pathways.³⁸ CCAT1 helped cell proliferation, growth, and mobility by targeting miR-181a-5p, and the silence of CCAT1 increased cell apoptosis.³⁶ In our study, we evaluated the diagnostic performance of CCAT1 and common routine markers for discriminating CRC cases from controls. We plotted the ROC curve, and CCAT1 was superior to the routine markers, CEA, and CA 19-9, for the diagnosis of CRC. Our results showed that CCAT1 was more sensitive (92.7%) with an AUC of 0.980 than serum CEA, which was 63.4% sensitive with an AUC of 0.757, and serum CA 19-9, which was 73.2% sensitive with an AUC of 0.665. Interestingly, our results matched those of Abedini et al., 2019, who reported that CCAT1 had a calculated AUC of 0.64 (95% CI: 0.811–0.94; $p = 0.024$), suggesting

a potential role for CCAT1 as a clinical biomarker with an indication of high discriminatory power.³⁹

When determining the diagnostic performance of CEA, CA 19-9 level, and plasma CCAT1 expression in detection of precancerous disease by the ROC curve analysis, we found that their diagnostic value was resolved using the calculated cutoff point of 1 ng/ml, 5.34 U/ml, and 1.37-fold change for CEA, CA 19-9, and CCAT1, respectively. The AUC was 0.506, 0.626, and 0.990 (the best), respectively. There was a limitation in this study due to the small number of subjects, which also limited the statistical power of some comparisons.

In conclusion, measuring the expression level of CCAT1 is more advised than tumor biomarkers such as CEA and CA 19-9 in patients with precancerous benign colorectal diseases for early diagnosis and prognosis of CRC. High expression of CCAT1 in CRC plasma may be used as a predictive biomarker for screening for CRC.

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Author Contributions

AAM, HOM, AASR and MIS, performed the laboratory work. AAM, AASR and MIS made the statistical analysis. MRS and MHA, examined the patients. AASR and MHA, collected samples. All authors participated in writing and reviewing the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Medical Ethics Committee of the Faculty of Medicine, Assiut University (dated June 2020).

Informed consent

A written informed consent was obtained from each participant before included in the study.

References

1. Rawla P, Sunkara T, & Barsouk A (2019). Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors *Gastroenterol Rev* 14(2) 89–103, <https://doi.org/10.5114/pg.2018.81072>.
2. Hassan A, Khalaf A, & Elias A (2021). Colorectal Cancer in Egypt: Clinical, Life-Style, and Socio-Demographic Risk Factors *Al-Azhar Int Med J*, <https://doi.org/10.21608/aimj.2021.79043.1490>.
3. Metwally IH, Abdelkhalek M, Elbalka SS, et al. (2019). Clinico-epidemiologic criteria and predictors of survival of rectal cancer among Egyptians in Delta region *J Coloproctology* 39(4) 339–345, <https://doi.org/10.1016/j.jcol.2019.07.004>.
4. Arhi C, Askari A, Nachiappan S, et al. (2021). Stage at Diagnosis and Survival of Colorectal Cancer with or without Underlying Inflammatory Bowel Disease: A Population-based Study *J Crohn's Colitis* 15(3) 375–382, <https://doi.org/10.1093/ecco-jcc/jjaa196>.
5. Marabotto E, Kayali S, Buccilli S, et al. (2022). Colorectal Cancer in Inflammatory Bowel Diseases: Epidemiology and Prevention: A Review *Cancers (Basel)*, <https://doi.org/10.3390/cancers14174254>.
6. Floreani A (2021). *Diseases of the liver and biliary tree* Dis Liver Biliary Tree, <https://doi.org/10.1007/978-3-030-65908-0>.
7. Ferrari A, Neefs I, Hoeck S, et al. (2021). Towards novel non-invasive colorectal cancer screening methods: A comprehensive review *Cancers (Basel)*, <https://doi.org/10.3390/cancers13081820>.
8. Kankanala VL, & SKR. M (2022). Carcinoembryonic Antigen - StatPearls - NCBI Bookshelf StatPearls [Internet], <https://doi.org/PMID: 35201700>.
9. Lee T, Teng TZJ, & Shelat VG (2020). Carbohydrate antigen 19-9 — tumor marker: Past, present, and future *World J Gastrointest Surg* 12(12) 468, <https://doi.org/10.4240/WJGS.V12.I12.468>.
10. Gao N, Li Y, Li J, et al. (2020). Long Non-Coding RNAs: The Regulatory Mechanisms, Research Strategies, and Future Directions in Cancers *Front Oncol*, <https://doi.org/10.3389/FONC.2020.598817>.
11. Xu MD, Qi P, & Du X (2014). Long non-coding RNAs in colorectal cancer: Implications for pathogenesis and clinical application *Mod Pathol* 27(10) 1310–1320, <https://doi.org/10.1038/modpathol.2014.33>.
12. Kishore C (2021). Epigenetic Regulation and Promising Therapies in Colorectal Cancer *Curr Mol Pharmacol*, <https://doi.org/10.2174/187446721466210126105345>.
13. Coppède F (2014). The role of epigenetics in colorectal cancer *Expert Rev Gastroenterol Hepatol*, <https://doi.org/10.1586/17474124.2014.924397>.
14. Su R, Wu X, Tao L, & Wang C (2022). The role of epigenetic modifications in Colorectal Cancer Metastasis *Clin Exp Metastasis*, <https://doi.org/10.1007/s10585-022-10163-w>.
15. Ghafari ABA-, Siddiqui HA, Choudhry HM, et al. (2022). Contribution of Long Non-Coding Rnas (Ccat1, Malat1, and Pandar) in the Pathogenesis of Colorectal Cancer *Pakistan J Med Heal Sci* 16(2) 512–517, <https://doi.org/10.53350/pjmhs22162512>.
16. Liu Z, Chen QJ, & Hann SS (2019). The functions and oncogenic roles of CCAT1 in human cancer *Biomed Pharmacother* 115(111) 108943, <https://doi.org/10.1016/j.biopha.2019.108943>.
17. Cancer.Net (2020). Colorectal Cancer: Stages | Cancer.Net *Am Soc Clin Oncol* 1–5.
18. Pfaffl MW (2001). A new mathematical model for relative quantification in real-time RT-PCR *Nucleic Acids Res* 29(9) e45, <https://doi.org/10.1093/NAR/29.9.E45>.
19. Sung H, Ferlay J, Siegel RL, et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries *CA Cancer J Clin* caac.21660, <https://doi.org/10.3322/caac.21660>.
20. Pellino G, Gallo G, Pallante P, et al. (2018). Noninvasive biomarkers of colorectal cancer: Role in diagnosis and personalised treatment perspectives *Gastroenterol Res Pract* 2018 1–21, <https://doi.org/10.1155/2018/2397863>.
21. Huang CS, Chen CY, Huang LK, et al. (2020). Prognostic value of postoperative serum carcinoembryonic antigen levels in colorectal cancer patients who smoke *PLoS One* 15(6) 1–14, <https://doi.org/10.1371/journal.pone.0233687>.
22. Hao C, Zhang G, & Zhang L (2019). *Serum CEA levels in 49 different types of cancer and noncancer diseases*, 1st ed Prog Mol Biol Transl Sci, <https://doi.org/10.1016/bs.pmbts.2018.12.011>.
23. Berkovich L, Gerber M, Katzav A, et al. (2022). NF-kappa B expression in resected specimen of colonic cancer is higher compared to its expression in

- inflammatory bowel diseases and polyps *Sci Rep* 12(1) 1–8, <https://doi.org/10.1038/s41598-022-21078-7>.
24. Vukobrat-Bijedic Z, Husic-Selimovic A, Sofic A, et al. (2013). Cancer Antigens (CEA and CA 19-9) as Markers of Advanced Stage of Colorectal Carcinoma *Med Arch (Sarajevo, Bosnia Herzegovina)* 67(6) 397–401, <https://doi.org/10.5455/medarh.2013.67.397-401>.
 25. Tümay V, & Guner OS (2020). The utility and prognostic value of CA 19-9 and CEA serum markers in the long-term follow up of patients with colorectal cancer. *Ann Ital Chir* (February) 19–22.
 26. Lakemeyer L, Sander S, Wittau M, et al. (2021). Diagnostic and Prognostic Value of CEA and CA 19-9 in Colorectal Cancer *Dis 2021, Vol 9, Page 21* 9(1) 21, <https://doi.org/10.3390/DISEASES9010021>.
 27. Subki AH, Alghamdi TA, Butt NS, et al. (2021). CEA and CA 19-9 Levels and KRAS Mutation Status as Biomarkers for Colorectal Cancer *Clin Oncol* 6 1802.
 28. Sánchez-Salcedo R, Miranda-Castro R, de-los-Santos-Álvarez N, et al. (2022). An electrochemical genosensing platform for the relative quantification of the circulating long noncoding RNA CCAT1 to aid in the diagnosis of colorectal cancer *Sensors Actuators B Chem* 376(November 2022) 132940, <https://doi.org/10.1016/j.snb.2022.132940>.
 29. Wang N, Yu Y, Xu B, et al. (2019). Pivotal prognostic and diagnostic role of the long non-coding RNA colon cancer-associated transcript 1 expression in human cancer (Review) *Mol Med Rep*, <https://doi.org/10.3892/mmr.2018.9721>.
 30. Slaby O (2016). Non-coding RNAs as biomarkers for colorectal cancer screening and early detection *Adv Exp Med Biol*, https://doi.org/10.1007/978-3-319-42059-2_8.
 31. Galamb O, Barták BK, Kalmár A, et al. (2019). Diagnostic and prognostic potential of tissue and circulating long non-coding RNAs in colorectal tumors *World J Gastroenterol* 25(34) 5026–5048, <https://doi.org/10.3748/wjg.v25.i34.5026>.
 32. Alaiyan B, Ilyayev N, Stojadinovic A, et al. (2013). Differential expression of colon cancer associated transcript1 (CCAT1) along the colonic adenoma-carcinoma sequence *BMC Cancer*, <https://doi.org/10.1186/1471-2407-13-196>.
 33. Nissan A, Stojadinovic A, Mitrani-Rosenbaum S, et al. (2012). Colon cancer associated transcript-1: A novel RNA expressed in malignant and pre-malignant human tissues *Int J Cancer* 130(7) 1598–1606, <https://doi.org/10.1002/ijc.26170>.
 34. Ghafouri-Fard S, Eghtedarian R, & Taheri M (2020). The crucial role of non-coding RNAs in the pathophysiology of inflammatory bowel disease *Biomed Pharmacother* 129(June) 110507, <https://doi.org/10.1016/j.biopha.2020.110507>.
 35. Ma D, Cao Y, Wang Z, et al. (2019). CCAT1 lncRNA Promotes Inflammatory Bowel Disease Malignancy by Destroying Intestinal Barrier via Downregulating miR-185-3p *Inflamm Bowel Dis* 25(5) 862–874, <https://doi.org/10.1093/ibd/izy381>.
 36. Shang A, Wang W, Gu C, et al. (2020). Long non-coding RNA CCAT1 promotes colorectal cancer progression by regulating miR-181a-5p expression *Aging (Albany NY)* 12(9) 8301–8320, <https://doi.org/10.18632/aging.103139>.
 37. Zhang R, Zhang G, Li B, et al. (2022). Analysis of LINC01314 and miR-96 Expression in Colorectal Cancer Patients via Tissue Microarray-Based Fluorescence In Situ Hybridization Dis Markers, <https://doi.org/10.1155/2022/5378963>.
 38. Xiong G, Feng M, Yang G, et al. (2017). The underlying mechanisms of non-coding RNAs in the chemoresistance of pancreatic cancer *Cancer Lett* 397 94–102, <https://doi.org/10.1016/j.canlet.2017.02.020>.
 39. Abedini P, Fattahi A, Agah S, et al. (2019). Expression analysis of circulating plasma long noncoding RNAs in colorectal cancer: The relevance of lncRNAs ATB and CCAT1 as potential clinical hallmarks *J Cell Physiol* 234(12) 22028–22033, <https://doi.org/10.1002/jcp.28765>.