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A Possible Role of Long Noncoding RNA CCAT2, Microrna -17 And C-MYC As Biological Markers in Colorectal Cancer and Inflammatory Bowel Disease Among Egyptian Patients

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Abstract

Colorectal cancer (CRC) is among the most common malignancies in humans. Crohn's disease (CD) and ulcerative colitis (UC) are part of the group of chronic inflammatory diseases known as IBD. The pathophysiology of IBD and the onset and progression of colorectal cancer are closely associated with abnormal expression of several coding and non-coding genes, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). The purpose of this study is to investigate the expression of lncRNA Colon cancer-associated transcript 2 (CCAT2), miRNA-17, and cellular myelocytomatosis gene (c-MYC) in patients with IBD and CRC to determine their potential value as noninvasive diagnostic biomarkers. This study included 120 participants, divided into four groups: UC, Crohn's disease, CRC, and healthy controls (30 each). All subjects underwent a comprehensive history, physical examination, laboratory tests, colonoscopy, and biopsy. Using real-time PCR, the levels of lncRNA CCAT2, miRNA-17, and c-MYC were assessed in serum samples. CCAT2, miRNA-17, and c-MYC expression levels were significantly higher in UC, CD, and CRC patients compared to controls. Furthermore, their expression levels showed a statistically significant difference across the IBD and CRC patient groups. In the UC group, the expression levels for CCAT2, miRNA-17, and c-MYC were upregulated compared to the control group. CCAT2 showed 98.9% sensitivity and 100% specificity, miRNA-17 showed 90% sensitivity and 100% specificity, and c-MYC showed 83.3% sensitivity and 93.3% specificity. For the CD group, the levels of CCAT2, miRNA-17, and c-MYC were likewise upregulated. CCAT2 exhibited 66.2% sensitivity and 93.3% specificity, miRNA-17 had 66.7% sensitivity and 100% specificity, while c-MYC showed 70% sensitivity and 100% specificity. In the CRC group, expression levels of CCAT2, miRNA-17, and c-MYC were all upregulated. CCAT2 showed 86.7% sensitivity and 100% specificity; miRNA-17 showed 86.7% sensitivity and 99.8% specificity; and c-MYC showed 83.3% sensitivity and 100% specificity. In conclusion, this study revealed that CCAT2, miR-17, and c-MYC may serve as noninvasive biomarkers for CRC and IBD in Egyptian patients.

Keywords: Colorectal cancer; IBD; CCAT2; miR-17; c-MYC

1. Introduction

Colorectal cancer (CRC) is the third prominent cause of cancer-related mortality globally. Globally, the incidence of colorectal cancer (CRC) among adults under 50 has increased in recent years. **[1]**. Reports of a rise in the occurrence of colorectal cancer in young adults date back to the 1990s in Egypt. Compared to the median age in Western nations, the median age of diagnosis is lower, ranging from 49 to 52 years **[2]**.

Numerous adaptable risk factors, including type II diabetes, sedentary lifestyles, obesity, and repeated use of antibiotics, are linked to the elevated incidence. The internal lining of the rectum, colon appendix and colon is where colorectal cancer starts. Contrasted to over 90% for stage I, about 60% of CRC patients are diagnosed with stage IV, or localized or distant metastases, which have poor prognoses and 5-year survival (12.5% to 70.4%). Therefore, these facts focus how crucial it is to create early molecular indicators for colorectal cancer **[3].**

CD and UC are two forms of inflammatory bowel disease (IBD), an immune-mediated inflammatory disease (IMID) affecting GIT. It is known that IBD is related to a multifactorial etiology that includes environmental, genetic factors, dysregulation of systemic immune function and host–microbial interactions in the environment in the gut **[4].** In Egypt and throughout Africa, prevalence and frequency seem to be increasing **[5].**

Although IBD inflicts a lot of suffering for the patient that results in many complaints, as increased risk of CRC, especially in UC patients. Prevention of IBD from developing into cancer is critical because this risk increases with the duration of the illness.

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Therefore, Understanding the detailed events that led to the development of IBD into cancer is crucial **[6].** There is an urge to develop a new reliable biomarker for convenient and better management of patients with IBD. After the publication of the results of IBD genome studies, there has been an increasing interest to analyze the relationship between IBD and microRNAs, which is one of the most important gene expression regulators **[7]**.

A family of genes known as long non-coding RNAs (lncRNAs) is entangled in human malignancies **[8].** Nowadays, a set of lncRNAs are involved in cancer progression, initiation and affection all hallmarks of malignancy. Nearly all cancer types have dysregulated lncRNAs, which control the epithelial to mesenchymal transition (EMT) of tumor cells, migration, propagation,and invasion, consequently promoting cancer growth **[9]**.

Thus, In the future, lncRNAs are thought to be promising new therapeutic targets. One of the lncRNAs that has been widely studied as an important regulator in the pathophysiology of tumour is colon cancer-associated transcript 2 (CCAT2). Cumulative research studies showed that CCAT2 exhibits a great functional versatility due to its direct interaction with transcription factors, numerous RNA binding proteins, and other non-coding RNA types, including microRNA **[10].** MicroRNAs (miRNAs) are considered non-coding RNA that can be investigated in diverse clinical samples, as stool, serum, and urine. Although miRNAs were initially recognized as having an impact on post-translational gene expression, research conducted in the past ten years has demonstrated that they are prospective biomarkers for the discovery of multiple types of malignancies **[11].**

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a crucial role in the regulation of gene expression by targeting messenger RNAs (mRNAs) for degradation or translational repression **[12]**.miRNAs are essential for healthy cell growth, maintenance, and other physiological functions. Dysregulated miRNAs play a role in the resistance to treatment, initiation, metastasis, and progression which makes them beneficial as therapeutic targets and clinical biomarkers for colorectal cancer **[13]**. MiR-92a, a member of the well-acknowledged oncomiR cluster miR-17-92, is one of the oncomiRs that is up-regulated in CRC **[3]**. The c-MYC gene encodes the MYC protein, which acts as a transcription factor for various cellular processes, such as differentiation, survival, metabolism, apoptosis, and proliferation. The c-MYC gene displays an essential role in the occurrence of colorectal cancer (CRC) and the progression of tumor genesis in a variety of malignant tumors **[14].**

Up to 70–80% of colorectal tumors had high c-MYC expression, which has been found to be aberrant in many human malignancies **[15]**. Expression of c-MYC, which controls miR-17, is up-regulated by CCAT2. This encourages the spread of cancer and causes genetic instability **[16]**. Current advanced research is investigating mimic biomarkers for a number of uses, such as customized medicine, medication development, disease diagnostics, and drug efficacy and toxicity. For example, scientists are creating mimic biomarkers for colorectal cancer (CRC) that can identify alterations in metabolic patterns, enabling early detection and treatment **[17]**.

TNM is the most commonly used staging system for CRC and is based on the depth of invasion of the bowel wall, the extent of regional lymph node involvement, and presence of distant sites of disease **[18]**:

Stage I: Cancer is confined to the colon or rectum wall.

Stage II: Cancer has grown through the wall but has not reached nearby lymph nodes.

Stage III: Cancer has spread to nearby lymph nodes.

Stage IV: Cancer has metastasized to distant organs.

In Egypt, the colorectal cancer (CRC) survival rate is comparatively poor. A study found that patients with colorectal cancer in Egypt had a median overall survival of approximately two years, with stage I patients living for forty-four months and stage IV patients living for only eight months. Several factors contribute to this low survival rate, one of which is that CRC is frequently detected in Egypt at advanced stages, especially in younger people. In actuality, people under 40 accounts for over one-third of CRC cases in Egypt, and these cases are frequently identified at more advanced stages **[19]**.

Our aim in this study is to ascertain the diagnostic value of lncRNA CCAT2, micro-RNA-17, and c-MYC as biomarkers in patients with CRC as well as early detection of CRC in patients with IBD.

2. Materials and methods

2.1. Subject selection

One hundred and twenty adults were involved in this prospective case-control study, which was determined using the sample size equation. All participants were selected randomly from the Tropical Medicine Department's outpatient clinics at the Faculty of Medicine, Kasralainy hospitals, and were subdivided equally into four groups: 30 UC patients (12 (40%) females and 18 (60%) males) with a mean age of 32.0±11.34 years, CD group: 30 CD patients (12 (40%) females and 18 (60%) males) with a mean age of 34.13±12.19 years, CRC group: 30 CRC patients (16 (53.3%) females and 14 (46.7%) males) with a mean age of 53.03±10.03 years and Control group: 30 healthy control subjects (14 (46.7%) females and 16 (53.3%) males) with a mean age of 41.93±7.20 years. Age > 18 years, a diagnosis of colorectal cancer (CRC) based

on many investigations as clinical, laboratory, endoscopic, and histological as well as history of ongoing infection were the inclusion criteria. Patients with other reasons for chronic diarrhea, those with a tumor other than CRC, those who were lactating, pregnant, or using estrogens for any cause, and those with a concurrent autoimmune or endocrine disease were excluded.

Every subject gave the informational consent for this study. The study protocol complied with the 1975 Helsinki Declaration's ethical principles and was accepted by Cairo University's Faculty of Medicine ethics committee.

2.2. Blood sampling

Each subject had 5.0 ml of venous blood drawn, and the separated serum was stored at -70°C. Laboratory tests were also performed, including erythrocyte sedimentation rate (ESR), serum albumin, C-reactive protein (CRP), and complete blood count (CBC).

2.3. Quantitative Real-time PCR (qPCR)

Relative quantitation expressions of lncRNA CCAT2, miRNA-17 and mRNA c-Myc were measured. miRNeasy mini kit was used for RNA extraction and purification of serum total RNA, including non-coding RNA (Qiagen, Valencia, CA, USA) according to manufacturer instructions. RNA samples were quantitated using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA). Reverse transcription (RT) was carried out on total RNA in a final volume of 20 uL RT reactions using the miScript II RT kit (Qiagen, Valencia, CA, USA) according to manufacturer instructions. Quantitative Real-time PCR (qPCR) for detection of lncRNA CCAT2 , miRNA- 17, and mRNA c-MYC was carried out using miScript SYBR® Green PCR kit and protocol for quantitative detection (Qiagen, Valencia, CA, USA) according to manufacturer instructions. SNORD 68, Cat No. MS00033712 was utilized (due to the lack of an endogenous reference housekeeping gene of miRNA in the serum). For the lncRNA CCAT2 and c-MYC in serum, GAPDH was employed as housekeeping gene. The primer sequences used for qPCR were as follow:

	c-MYC F:(5'to 3') GCC CAG TGA GGA TAT CTG GA
	c-MYC R:(5' to 3') ATC GCA GAT GAA GCT CTG GT
GAPDH	(QT 300079247) F: (5'to 3') TGAAGGTCGGAGTCAACGGATTTGGT
	R :(5' to 3') CATGTGGGCCATGAGGTCCACCAC

The primers used are Hs-miR-17, Cat No. (MS00006524), and CCAT2, Cat No. (LP 01147A) and GAPDH QT 300,079,247 (as the endogenous housekeeping gene).

miR-17, CCAT2 and c-MYC expression levels were measured using the equation $2^{-\Delta\Delta Ct.}$ ΔΔCt was calculated by subtracting the ΔCt of the control samples from the ΔCt of the disease samples.

2.4. Colonoscopy

Colonoscopy, with biopsy and histological examination were done for confirmation of the diagnosis of CRC **[20].** Colonoscopy and biopsy were done for IBD patients: to confirm diagnosis as well as assessing disease activity and extent. Crohn's disease activity index (CDAI score) for CD was evaluated. Remission was considered when the score below 150, 150–219 was considered moderate, and >450 to be severe. UC Mayo score: < 2 is in remission, 3-5 are considered mild, 6-10 are considered moderate and 11-12 are severe.

2.5. Statistical analysis

Statistical package of social science (SPSS 17.0) on windows 8.1was used to analyze our results. For comparing 2-independent groups ANOVA test one was used and the other for more than 2-independent groups. To test significance, Benferroni Post-Hoc was used. For the non-parametric data in comparing more than 2-independent groups Kruskalwallis and Mann-whitney test were used. Bivariate Pearson correlation test with a two-tailed to test the significance was used. Sensitivity and specificity test were generated for testing a new test with ROC Curve (Receiver Operating Character). P-value<0.05 was considered as a cutoff value for significance **[21].**

2.6. Sample size calculation

Sample size calculation for CRC patients

$$N = \frac{Z_{1-\frac{\alpha}{2}}^{2}\, p(1-p)}{d^2} = 28.2$$

Where N is the Sample size, $Z_{1-\frac{\alpha}{2}}$ is the standard normal variant with p-value <0.05, p is the expected proportion in population based on previous studies. p=0.015% (1.2 million person per 7.97 billion person worldwide), d is the absolute error/precision. d=3.2%, A confidence level of 95% and the threshold of significance is 0.05. Therefore, a minimum of 29 persons would be required to be included in the study **[22 - 24]**

Sample size calculation for UC patients

$$N = \frac{Z_{1-\frac{\alpha}{2}}^2\, p(1-p)}{d^2} = 29.8$$

Where N is the Sample size, $Z_{1-\frac{\alpha}{2}}$ is the standard normal variant with p-value <0.05, p is the expected proportion in population based on previous studies and the distribusion of UC at diagnosis. p=64% (6,678 person per 10,400 persons), d is the absolute error/precision. d=12.5%., A confidence level of 95% and the threshold of significance is 0.05.

Therefore, a minimum of 30 persons would be required to be included in the study **[22] and [25].**

Sample size calculation for CD patients

$$N = \frac{Z_{1-\frac{\alpha}{2}}^2\, p(1-p)}{d^2} = 28.14$$

Where N is the Sample size, $Z_{1-\frac{\alpha}{2}}$ is the standard normal variant with p-value <0.05, p is the expected proportion in population based on previous studies and the distribusion of CD at diagnosis. p=34% (3,495 person per 10,400 persons), d is the absolute error/precision. d=12.5%, A confidence level of 95% and the threshold of significance is 0.05.

Therefore, a minimum of 29 persons would be required to be included in the study **[23] and [24].**

3. Results

3.1. Demographic characteristics and clinical data of the study groups

Most of the recruited patients were between thirties to fifties as shown in Table (1). Some descriptive and personal history data was considered in the study such as smoking, coffee consumption, diabetes and hypertension were illustrated in Table (2). There was no statistical significance between the groups as regards the descriptive data (p>0.05). Meanwhile, there is a statistically significant difference between CRC and the other groups regarding smoking and diabetes (p<0.05).

Table (1): The Age & Gender classifications of all the studied groups

Parameters	UC (N=30)	CD (N=30)	CRC (N=30)	control(N=30)	P-value
Age **(years)**	32.0±11.34	34.13±12.19	53.03±10.03	41.93±7.20	0.66[a], **0.04[b],** 0.34[c], **0.041[d],** 0.16[e], 0.89[f]
Gender *Male* **n (%)** *Female* **n (%)**	 18 (60%) 12 (40%)	 18 (60%) 12 (40%)	 14 (46.7%) 16 (53.3%)	 16 (53.3%) 14 (46.7%)	1.00

Age is shown as mean ±SD, Gender is presented by n (%). p-value: a between UC and CD, b between UC and CRC, c between UC and control, d between CD and CRC, e between CD and control, f between CRC and control. Bold is significant **(P≤0.05)**

Table (2): Descriptive and personal history for the studied groups

Parameters	UC (N=30)	CD (N=30)	CRC (N=30)	Control (N=30)	p-value
Smoking					
Smoker	7 (23.3%)	9 (30%)	21(**70%**)	3 (9.9)	**<0.05**
Non-smoker	23 (76.7%)	21 (70%)	9 (**30%**)	27 (90.1%)	
Coffee consumption					
Yes	6(20%)	11 (36.7%)	10 (33.3%)	3 (9.9)	>0.05
No	24 (80%)	19 (63.3%)	20 (66.7%)	27 (90.1%)	
Diabetes					
Yes	4 (13.3%)	1 (3.3%)	15(**50%**)	2 (6.7)	**<0.05**
No	26(86.7%)	29 (96.7%)	15(50%)	28 (93.3%)	
Hypertension					
Yes	1 (3.3%)	1 (3.3%)	2 (6.7)	3 (9.9)	>0.05
No	29 (96.7%)	29 (96.7%)	28 (93.3%)	27 (90.1%)	

Data shown as N (%) Chi-squared Test is used, Mann-Whitney U Test for significance. * Significant at p≤0.05 between CRC group and all other groups.

3.2. Laboratory investigations of the studied groups

As shown in Table (3), Hb levels in the CRC group were considerably lower than those in the UC, CD, and control groups (p values = 0.013, 0.002, and 0.014, respectively), and in the UC group than in the control

group (p = 0.047). No significant differences were seen between the UC and CD patient groups. All investigated groups had considerably higher CRP levels than the control group (p =0.0001), and the differences between CRC and UC and CD (p =0.013 and 0.001), as well as between CD and UC (p =0.001), were highly significant. In comparison to the control group, the UC, CD, and CRC groups exhibit significantly increased ESR levels. The albumin was statistically significant lower in UC, CD & CRC compared to control.

Table (3): Laboratory investigations of all studied groups

Tests	UC (N=30)	CD (N=30)	CRC (N=30)	control (N=30)	p-value
Hb (g/dl)	11.28±2.07	11.83±1.88	10.92±2.70	12.32±1.38	0.53a, **0.013b**, **0.047c**, **0.002d,** 0.18e, **0.014f**
TLC ($x10^3$/uL)	8.38±5.09	8.55±4.24	6.81±2.47	7.78±2.42	0.82a, 0.116b, 0.069c, 0.05d, 0.047e, 0.572f
Platelet count ($x10^3$/uL)	307±110.4	363.2±128.2	265.4±91.5	286.57±58.12	0.34a, 0.57b, 0.019c, 0.001d, 0.001e, 0.03f
CRP (mg/L)	14.69±16.9	31.52±42.5	45.0±35. 15	4.69±1.6	**0.001a,0.013,b 0.0001c,0.001d ,0.0001e,f**
ESR (mm/h)	32.47±27.5	35.73±28.4	46.0±33.95	14.37±6.06	0.956a, 0.096b, **0.047c**, 0.360d, **0.03e**, **0.001**f
Albumin (g/dL)	3.77±0.81	3.51±0.76	3.63±0.459	4.41±0.24	0.661a, 0.91b, **0.0001c**, 0.31d, **<0.0001e,** f

Data shown as mean ±SD, Independent sample T-tests used . * significant (P≤0.05) a between UC and CD, b between UC and CRC, c between UC and control, d between CD and CRC, e between CD and control, f between CRC and control.

3.3. Serum levels of CCAT2, miR-17 and c-MYC among the studied groups

The estimated expression levels of all biomarkers show high statistical significance compared to healthy controls (Table 4). CCAT2 was highly significant in UC, CD & CRC compared to control (p-value <0.0001, 0.001 & <0.0001 respectively), also a statistically significant difference is shown between UC & CD (p-value 0.0001) and between each of UC & CD compared to CRC (p-value 0.027, 0.0001 respectively). For miR-17,a high statistical significance difference is detected in UC, CD & CRC in comparison to control (p-value <0.0001, 0.0001 & <0.0001 respectively), and between UC & CD (p-value 0.010), no statistical difference between UC & CRC is observed but there was a statistically significant difference between CD & CRC (p-value 0.036).

Regarding c-MYC, a statistically significant difference was found between UC, CD & CRC and control (p-value <0.0001, 0.0001 & <0.0001 respectively), no statistical difference between UC & CD, but by comparing UC & CD to the CRC group, a statistically significant difference was found (p-value 0.006 & 0.004 respectively).

Table (4): The relative expression levels of CCAT2, miR-17 and c-MYC biomarker among the studied groups

Biomarkers	UC (N=30)	CD (N=30)	CRC (N=30)	control(N=30)	P-value
CCAT2(log2)	6.72±2.96	2.04±1.70	4.89±3.27	1.01±0.05	**0.0001^a,0.027^b, <0.0001^c, 0.0001^d, 0.001^e, <0.0001^f**
miR-17(log2)	5.05±4.19	1.73±1.21	4.16±2.91	1.01±0.05	**0.010^a,** 0.345^b, **<0.0001^c, 0.036^d, 0.0001^e, <0.0001^f**
c-MYC(log2)	2.68±1.69	2.56±1.80	4.38±2.74	1.01±0.05	0.70^a**, 0.006^b, <0.0001^c, 0.004^d, 0.0001^e, <0.0001^f**

Data shown as mean ±SD, T-tests was used. * Significant (P≤0.05) a between UC and CD, b between UC and CRC, c between UC and control, d between CD and CRC, e between CD and control, f between CRC and control.

3.4. Relationship between serum biomarkers CCAT2, miR-17 and c-MYC and the severity of the disease in the UC and CD groups

Tables (5, 6), show that CCAT2 expression levels were increased as the disease got worse, according to a correlation between the expression levels of CCAT, miR-17, and c-MYC in the group of UC patients. On the other hand, miR-17 levels tended to elevate in patients who were in remission activity and exhibited a significant difference between the groups (p-value 0.013 between remission and mild cases, p-value 0.001 between remission and moderate, and p-value 0.001 between remission and severe). c-MYC levels were not shown to be significant, although they did tend to rise as severity increased.

Regarding CD group, CCAT2 level tended to highly increase in remission and mild cases, the level of miR-17 tended to be higher in patients with remission activity, miR-17 tended to elevate in severe patients as

well. No significance was detected as regards to the activity and c-MYC level but also the level tended to elevate in CD patients with severe activity.

Table (5): The relative expression level of CCAT2, miR-17 and c-MYC in UC patients as regards disease severity

Parameters	Remission (N=2)	Mild (N=9)	Moderate (N=9)	Severe (N=10)
CCAT2(log2)	4.93±3.32	5.99±2.03	6.66±2.56	7.93±3.99
p-value=0.389[a], 0.574[b], 0.050[c], 0.095[d], 0.375[e], 0.254[f]				
miR-17(log2)	9.89 (0.108-19.68)	2.66 (0.33-15.12)	4.18 (0.75-8.79)	5.01 (2.39-9.03)
p-value=0.013[a], 0.001[b],0.001[c], 0.278[d], 0.070[e], 0.145[f]				
c-MYC(log2)	2.32±1.60	2.14±1.85	2.45±1.44	3.44±1.73
p-value=0.569[a], 0.977[b], 0.814[c], 0.327[d],0.645[e], 0.646[f]				

Data shown as mean ±SD, T-tests were used for CCAT and C-Myc. For miR-17: Data shown as Median (Range). Chi-squared Test is used. Mann-Whitney U Test for significance. * Significant (P≤0.05). a between Remission and Mild, b between Remission and Moderate, c between Remission and Severe, d between Mild and Moderate, e between Mild and Severe, f between Moderate and Severe.

Table (6): Relationship between serum biomarkers and the severity of the disease in CD group

Parameters	Remission (N=5)	Mild (N=5)	Moderate (N=16)	Severe (N=4)
CCAT(log2)	2.70±2.3	2.60±1.67	1.71±1.68	1.86±0.91
p-value=0.606[a], 0.622[b], 0.253[c], 0.750[d], 0.334[e], 0.162[f]				
miR-17 (log2)	3.56±2.76	2.26±2.10	2.27±1.57	2.74±2.33
p-value=0.224[a], 0.468[b], 0.35[c], 0.453[d], 0.631[e], 0.191[f]				
C-MYC(log2)	2.47±1.73	2.87±1.61	2.17±1.71	3.81±2.52
p-value=0.981[a]0.572[b], 0.633[c], 0.572[d], 0.620[e], 0.736[f]				

Data shown as mean ±SD, T-test was used. * Significant (P≤0.05). a between Remission and Mild, b between Remission and Moderate, c between Remission and Severe, d between Mild and Moderate, e between Mild and Severe, f between Moderate and Severe.

3.5. ROC curve to evaluate the diagnostic and prognostic performances of CCAT2, miR-17 and c-MYC in the study groups

Figure 1 shows the ROC curve in UC patients, in which CCAT2 has a cut-off level of 2.57, with 99.9% Sensitivity and 100% Specificity (AUC, area under curve = 0.999, p-value **<0.0001**). miR-17 has a cut-off level of >1.2, 90% Sensitivity and 100% Specificity (AUC = 0.900, p-value **<0.0001).** c-MYC has a cut-off value of 1.69, with 83.3% sensitivity and 93.3% specificity (AUC = 0.829, p-value**<0.0001).**

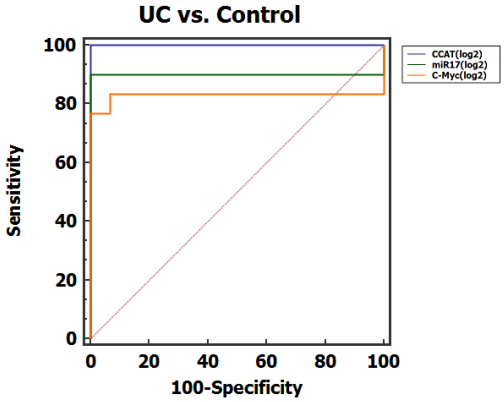

Figure (1): ROC Curve for CCAT2, miR-17 and c-MYC in serum for patients with UC

Figure 2 shows the ROC in CD patients, in which CCAT2 has a cut-off level of 1.7, 66.2% with 93.3% Sensitivity and Specificity (AUC, area under curve = 0.662, p-value **0.05**). miR-17 has a cut-off level of >1.21, 66.7% Sensitivity and 100% Specificity (AUC = 0.667, p-value **0.05).** c-MYC has a cut-off value of 1.8, with 70% sensitivity and 100% specificity (AUC = 0.700, p-value **0.0168**

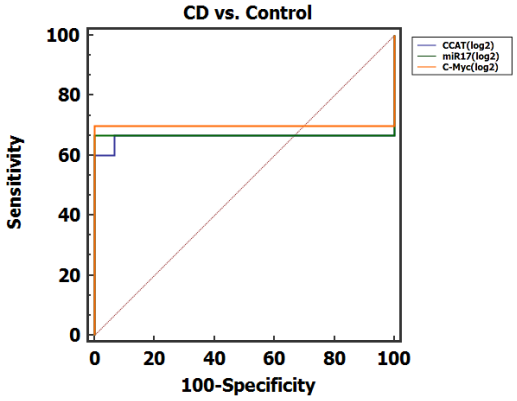

Figure (2): ROC Curve for CCAT2, miR-17 and c-MYC in serum for patients with CD

Figure 3 shows the ROC in CRC patients, in which CCAT2 has a cut-off level of 2.68, with 86.7% Sensitivity and 100% Specificity (AUC, area under curve = 0.867, p-value **<0.0001**). .miR-17 has a cut-off level of >2.98, 86.7% Sensitivity and 99.8% Specificity (AUC = 0.867, p-value **<0.0001).** c-MYC has a cut-off value of >2.75, with 83.3% sensitivity and 100% specificity (AUC = 0.833, p-value **<0.0001).**

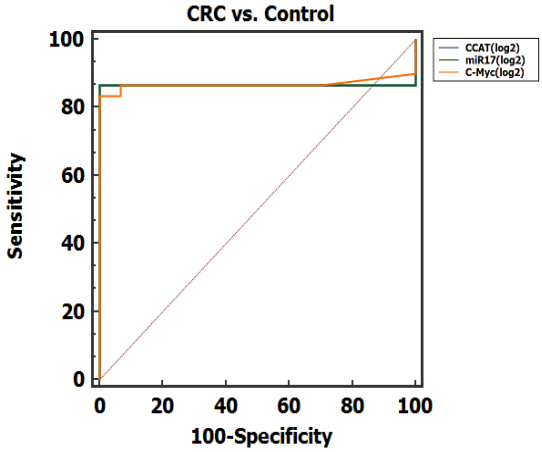

Figure (3): ROC Curve for CCAT2, miR-17 and c-MYC in serum for patients with CRC

According to the above values ROC analysis indicated the diagnostic efficacy of the studied biomarkers in discriminating CRC patients from controls.

The expression level of CCAT2 in males tended to have higher level of CCAT2 than females with significant difference between both genders (p=0.010) among CRC patients. Regarding the anatomical site CCAT2 expression level, there were significant differences between patients affected at Colon and Rectal (p=0.05); between Colon and rectosigmoid (p=0.03) and between Sigmoid and rectosigmoid patients (p=0.026). Both rectosigmoid and rectal had higher levels of CCAT2 than other sites. Also, for the stages of tumor, there were a high significant difference between (Stage III, Stage IV) and (Stage I, Stage II) with p-value 0.05. In general Adenocarcinoma patients had higher levels of CCAT2 than Mucoid with mean and standard deviation of 4.93±3.41 and 4.56±2.08 respectively. Regarding CT Analysis, results showed that patients with Wall Thickening had lower levels of CCAT2 (p value=***0.016)*** in addition the presence of liver metastases has a statistically significant difference decrease (p=0.018) in the expression level of CCAT2 when compared to its absence, while no statistically significant difference is detected in the involvement of LNs. **Table (7)**

Table (8) demonstrates that there was significant difference between the serum biomarker and the anatomical site (between Colon and Rectal and between Colon and Sigmoid) with p-values 0.004 and 0.05 respectively. Also, for the stages of tumor, there were a highly significant difference between (Stage III, Stage IV) and (Stage I, Stage II) with p-value 0.005. In general Adenocarcinoma patients had higher levels of miR-17 than

Mucoid with mean and standard deviation of 4.27±2.95 and 3.24±2.91 respectively at p-value of 0.001. Also reveals a statistically significant increase in the level of miR-17 in individuals with localized LNs and liver metastases (p=0.006 and 0.043, respectively).

Table (7): CCAT2 expression level among CRC group regarding gender, anatomical site, tumor types and CT analysis

Parameters	CRC (N=30)	
	CCAT2(log2)	p-value
Gender		
Female (N=17)	3.56±2.74	**0.010***
Male (N=13)	6.64±3.17	
Anatomical site		
Colon (N=10)	3.91±3.68	**0.05[a]**, 0.228[b], **0.030[c]**, 0.214[d], 0.215[e], 0.60[f], 0.154[g], **0.026[h]**, 0.831[i], 0.280[j]
Rectal (N=10)	5.76±3.28	
Sigmoid (N=5)	3.23±2.63	
Rectosigmoid (N=2)	8.00±1.19	
Cecum (N=3)	6.0±2.15	
Tumor Types		
Adenocarcinoma (N=27) 4.93±3.41		
Stage I, Stage II (N=12)	3.24±1.02	**0.05[x], 0.018[y]**
Stage III, Stage IV (N=15)	6.28±2.70	
Mucoid (N=3) 4.56±2.08		
CT Analysis		
Wall Thickening		
Absent (N=17)	6.12±2.84	**0.016***
Present (N=13)	3.29±3.20	
Regional LNs		
Absent (N=15)	5.54±3.37	0.720
Present (N=15)	4.24±3.15	
Liver Metastasis		
Absent (N=26)	5.10±3.43	**0.018***
Present (N=4)	3.52±1.69	

Data shown as mean ±SD, Independent sample T-tests used .a between Colon and Rectal; b between Colon and Sigmoid; c between Colon and Rectosigmoid; d between Colon and Cecum; e between Rectal and Sigmoid; f between Rectal and Rectosigmoid; g between Rectal and Cecum; h between Sigmoid and Rectosigmoid; i between Sigmoid and Cecum; j between Rectosigmoid and Cecum. x between Adenocarcinoma and Mucoid, y between (Stage I, Stage II) and (Stage III, Stage IV) . * Significant (P≤0.05)

Table (8): Relative expression level of miR-17 among CRC group regarding, anatomical site, tumor types and CT analysis

Parameters	CRC (N=30)	
	miR-17(log2)	p-value
Anatomical site		
Colon (N=10)	4.85±4.06	**0.044[a]**, **0.050[b]**, 0.172[c], 0.053[d], 0.696[e], 0.766[f], 0.435[g], 0.969[h], 0.552[i], 0.398[j]
Rectal (N=10)	4.03±2.50	
Sigmoid (N=5)	3.84±2.11	
Rectosigmoid (N=2)	4.58±2.26	
Cecum (N=3)	2.61±1.58	
Tumor Types		
Adenocarcinoma (N=27) 4.27±2.95		
Stage I, Stage II (N=12)	2.39±1.81	**0.005[x], 0.001[y]**
Stage III, Stage IV (N=15)	5.78±2.84	
Mucoid (N=3) 3.24±2.91		
CT Analysis		
Regional LNs		
Absent (N=15)	3.39±1.92	**0.006***
Present (N=15)	4.94±3.54	
Liver Metastasis		
Absent (N=26)	3.84±2.50	**0.043***
Present (N=4)	6.27±4.79	

Data shown as mean ±SD, Independent sample T-tests used .a between Colon and Rectal; b between Colon and Sigmoid; c between Colon and Rectosigmoid; d between Colon and Cecum; e between Rectal and Sigmoid; f between Rectal and Rectosigmoid; g between Rectal and Cecum; h between Sigmoid and Rectosigmoid; i between Sigmoid and Cecum; j between Rectosigmoid and Cecum. x between Adenocarcinoma and Mucoid, y between (Stage I, Stage II) and (Stage III, Stage IV) . * Significant (P≤0.05)

As seen in **Table (9)** the c-MYC expression level in the CRC group, shows significant difference in respect to the anatomical site between (rectal and sigmoid, rectal and rectosigmoid, sigmoid and cecum and between rectosigmoid and cecum with p values (**0.014, 0.036, 0.05, and 0.012)** respectively. Also, there was a high significant difference between (Stage III, Stage IV) and (Stage I, Stage II) with p-value **0.032** for both. In general, Adenocarcinoma patients had higher levels of c-MYC than Mucoid, p-value of **0.014**. Meanwhile, there is no statistically significant difference in c-Myc expression levels across CRC group in terms of regional LNs or liver metastasis.

Table (9): Expression level of c-MYC among CRC group regarding, anatomical site, tumor types and CT analysis

Parameters	**CRC (N=30)**	
	C-MYC(log2)	**p-value**
Site of Anatomy		
Colon (N=10)	4.89±2.29	0.088[a], 0.456[b], 0.065[c], 0.307[d], **0.014[e], 0.036[f]**, 0.345[g], 0.656[h], **0.05[i],0.012[j]**
Rectal (N=10)	3.29±1.59	
Sigmoid (N=5)	6.25±4.89	
Rectosigmoid (N=2)	3.51±4.0	
Cecum (N=3)	3.84±1.01	
Tumor Types		
Adenocarcinoma (N=27) 4.53±2.82		
Stage I, Stage II (N=12)	3.10±2.22	**0.032[x], 0.014[y]**
Stage III, Stage IV (N=15)	5.66±2.78	
Mucoid (N=3) 3.11±1.77		
CT Analysis		
Regional LNs		
Absent (N=15)	3.93±3.38	0.092
Present (N=15)	4.83±1.96	
Liver Metastasis		
Absent (N=26)	4.36±2.85	0.644
Present (N=4)	4.50±2.24	

Data shown as mean ±SD, Independent sample T-tests used .a between Colon and Rectal; b between Colon and Sigmoid; c between Colon and Rectosigmoid; d between Colon and Cecum; e between Rectal and Sigmoid; f between Rectal and Rectosigmoid; g between Rectal and Cecum; h between Sigmoid and Rectosigmoid; i between Sigmoid and Cecum; j between Rectosigmoid and Cecum. x between Adenocarcinoma and Mucoid, y between (Stage I, Stage II) and (Stage III, Stage IV) .

4. Discussion

Finding noninvasive methods for cancer diagnosis is a goal shared by many researchers. Three genes were evaluated in patients with CRC and IBD in the current investigation. These genes were chosen based on the theory that CCAT2 causes genomic instability, increases the risk of cancer spreading, and controls miR-17 via upregulating c-MYC expression **[26].**

As far as we are aware, this is the first molecular study using serum samples to investigate the potential significance of Lnc RNA CCAT2, microRNA-17, and c-Myc as biological markers in colorectal cancer patients to aid in early diagnosis of CRC in inflammatory bowel disease (IBD) patients. CRC mortality can be considerably decreased with early detection and prognosis prediction **[27].** Our results demonstrated that CRC patients are much older than those in the UC and CD patient categories. Our results agree with those of **Abdel-Hamid et al.,** who reported that research conducted in Egypt and other Arab nations revealed an increased incidence of CRC in individuals over the age of forty **[28].** There was no statistical significance regarding Gender differences among the studied groups. Contrary to what **Abancens et al.,** found that men had a greater incidence of CRC than women do **[29].**

Regarding CRC, smoking and diabetes, the difference between CRC and the other groups is statistically significant. **Paul et al.,** found a 1.3-fold higher incidence of CRC in people with type 2 diabetes **[30].** These results are consistent with ours. Our findings agreed with those of **Mosli et al.,** who found that essential hypertension, iron deficiency anemia, and diabetes mellitus were the most prevalent comorbidities **[31].** According to **Elbadry et al.,** stated that diabetes mellitus (DM) and hypertension (HTN) were the most prevalent comorbid conditions among patients **[32].** Our results regarding smoking are consistent with those of **Botteri et al.,** who found that risk increases with duration and smoking intensity compared to nonsmokers. Compared to non-smokers, current smokers had a greater risk for the developing of CRC **[33].**

By promoting gut microbial dysbiosis, which affects metabolites in particular taurodeoxycholic acid (TDCA), cigarette smoking may exacerbate colorectal cancer. Elevated gut TDCA may trigger the colon epithelium's oncogenic MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated protein kinase 1/2) pathway, which in turn may promote the growth of colonocytes. Additionally, smoking cigarettes may impair the function of the gut barrier, which makes it easier for TDCA to trigger colonic oncogenic MAPK/ERK activation **[34]**.

It is yet unclear what the underlying processes of the possible association between diabetes mellitus and colorectal cancer are. Insulin is a powerful growth agent that either directly or indirectly promotes cell division and carcinogenesis through insulin-like growth factor 1 (IGF-1). Because hyperinsulinemia inhibits IGF binding protein-1, it increases the bioactivity of IGF-1. Since hyperglycemia stimulates the multiplication of cancer cells both directly and indirectly, it provides a plausible explanation for carcinogenesis. Chronic inflammation is also thought to be a sign of carcinogenesis **[35].**

The present study showed that Hb levels were considerably lower in CRC. These findings were supported by **Calleja et al.,** who stated that anemia is the most prevalent symptom, arising in 30–75% of patients with CRC **[36].** They also aligned with the findings of **Almilaji et al.,** who established that iron deficiency anemia is frequently observed in CRC patients **[37].**

The current study revealed that CRP levels were significantly higher in all studied groups compared to the control and were highly significant in CRC when compared to UC, and CD), as well as in CD compared to UC. These results align with **Jeong et al.,** who found that UC patients' CRP levels were noticeably greater than those of healthy control groups **[38],** and with a study done by **Holm et al.,** who recruited 19 CRC patients displaying a high CRP level, which is associated with a poor prognosis **[39].**

As regards albumin levels in CD and CRC, Compared to individuals in the control group, it was noticeably lower. This supports research by **Park et al.,** showing that albumin has specific antioxidant properties and may be affected in IBD and other inflammatory conditions due to proteinuria, which is intensely related to the development of CD**[40]. Tang et al.,** conducted a study that showed higher prediction of cancer mortality with lower serum albumin levels **[41].**

Regarding the activity of UC, **Mortensen et al.,** found that 90% of the UC group had an ongoing disease, with 10% being in remission. Their results concur with our findings **[42].Chen et al.,** found, in contrast to our results, that the UC group had a higher proportion of remission (35.6%). For CD group, both discovered that 42% and 49.8% of the CD group, respectively, were in remission **[43].**

The current study found that the colon and rectum were the most affected regions in CRC (33.3%) each. These results are in consistent with a study by **Jurescu et al.,** on colorectal cancer (CRC) patients which discovered (32.4%) in the left colon, (21.1%) in the right colon, , and (46.5%) in the rectum **[44].**Our analysis revealed that only 10% of the patients had mucoid type, while 90% of them had adenocarcinoma at various stages. As per the **American Cancer Society**, most colorectal cancers are adenocarcinomas. This finding is in line with our findings **[45].**

As regards the molecular markers, our results showed that in the UC group, the expression levels for CCAT2, miRNA-17, and c-MYC were upregulated compared to the control group. CCAT2 showed 98.9% sensitivity and 100% specificity, miRNA-17 showed 90% sensitivity and 100% specificity, and c-MYC showed 83.3% sensitivity and 93.3% specificity. For the CD group, the levels of CCAT2, miRNA-17, and c-MYC were likewise upregulated. CCAT2 exhibited 66.2% sensitivity and 93.3% specificity, miRNA-17 had 66.7% sensitivity and 100% specificity, while c-MYC showed 70% sensitivity and 100% specificity. In support of our findings on c-MYC in IBD, **Macpherson et al.,** collected biopsies from 34 patients with known or suspected IBD. They found that c-MYC mRNA levels were higher in colonoscopy samples from active regions of inflammatory bowel disease than in inactive regions in the same patient. In the CRC group, expression levels of CCAT2, miRNA-17, and c-MYC were all upregulated. CCAT2 showed 86.7% sensitivity and 100% specificity; miRNA-17 showed 86.7% sensitivity and 99.8% specificity; and c-MYC showed 83.3% sensitivity and 100% specificity**[46].** in agreement with our results, **Zhang et al.,** and **Gao et al.,** discovered that CRC tissues had significantly higher levels of lncRNA CCAT2 expression in comparison to adjacent noncancerous mucosa tissues**[47, 48].**

Our findings on miR-17 in CRC patients were consistent with those of **Fu et al.,** and **Huang et al.,** who revealed that patients with colorectal cancer had considerably higher levels of miR-17 expression, which could indicate a bad prognosis. In keeping with our findings **[49, 50]**, **Yu et al.,** assessed the expression of miR-17 in 47 pairs of CRC tissues that have marked elevation of miR-17 level **[51].**As regards c-MYC upregulation, our results were in accordance with **Hartman et al.,** who discovered that IBD-associated intestinal adenocarcinomas have distinct molecular alterations, one of which is c-MYC amplification, which occurs in one-third of cases with CD and UC backgrounds **[52].**

Furthermore, the current study found a positive correlation between c-MYC levels in UC patients' serum and ESR, **Sipos et al.,** reported that the c-MYC gene is dysregulated in inflammation and overexpressed in colon adenocarcinomas **[53].** Based on the severity of our patients, our research indicates a correlation between the degree of CCAT2, miR-17, and c-MYC expression with the severity of the condition. **Ayoub et al.**, evaluated the degree of the same Lnc-RNA expression in ulcerative colitis and its asscociation with the illness's severity, partially agreed with our findings, highlighted the possibility of using it as a biomarker for UC diagnosis and prognosis **[54].**

The tumor staging analysis's findings demonstrated that CCAT2 expression levels increased with tumor stage. The results of the tumor staging analysis showed that stages III and IV have considerably higher levels than stages I and II .Supporting our findings is the finding by **Xu et al.,** who showed that higher TNM stage in hepatocellular cancer was associated with greater expression of CCAT2 **[55]**, and by **Cai et al.,** who showed that the levels of CCAT2 were significantly elevated in high-grade pancreatic ductal adenocarcinoma tissues **[56].** In both metastasis-positive and metastasis-negative individuals, CCAT2 and c-Myc were markedly up-regulated in the tumors relative to matched nontumorous tissues ($p < 0.0001$). As the expression of CCAT2 is connected with c-MYC, indicating that CCAT2 may contribute to metastasis through its regulation of c-MYC, the significant correlation between metastasis and the expression of c-Myc in the tumors may account for the higher levels of CCAT2 in advanced stages of colorectal cancer **[57].**

In addition, stages III and IV have significantly higher levels of miRNA-17 than stages I and II. This is consistent with research by **Hussen et al.,** who found a correlation between the miRNA expression profile and the detection, staging, and progression of human malignancies **[58].** Likewise, c-MYC level showed a highly significant difference between (Stage III, Stage IV) and (Stage I, Stage II). These findings agree in part with **Lian et al.,** who showed that in esophageal squamous cell carcinoma, there was a substantial correlation between c-MYC expression and clinical stage **[59].**

CRC patients underwent CT scanning to establish a connection between localized LNs and liver metastases and the expression levels of CCAT2, miRNA-17, and c-MYC. CCAT2 levels were lower in patients with liver metastases, but the involvement of LNs did not differ statistically significantly. **Franz et al.**, discovered increased expression of CCAT2 in colorectal liver metastases, which is contrary to our findings **[60].** According to **Tan et al.,** patients with high CCAT2 expression were more prone to develop lymph node metastases **[61]**.For miR-17, **Lai et al.,** and **Yu et al.,** demonstrated that CRC tissues and lymph node metastases had significantly higher levels of miR-17, which is in line with our findings **[62, 63]**.

However, there is no statistically significant difference in c-MYC expression levels among CRC group regarding regional LNs or liver metastasis. This contrasts with **Yang et al.,** study, which measured c-MYC in sections of metastasizing and non-metastasizing human CRC and found that the expression of c-MYC showed higher statistical significance in the metastatic group compared to non-metastasizing CRC **[64].**

Moreover, **Siddiqui et al.,** and **Xing et al.,** detected that CCAT2 causes up-regulation of expression c-MYC, which regulates miR-17. This increases the risk of cancer spreading and causes genetic instability **[65, 66]**. This is in agreement with a **2013** study by **Ling et al**. that showed CCAT2 upregulates miR-17 and c-MYC. Both investigations validate our findings **[67]**.

Its functions as an activator of the WNT/β-catenin pathway, enhancer of MYC expression, regulator of the TCF7L2 transcription factor, and contributor to chromosomal instability are its definitive mechanisms **[68]**.Although rectosigmoid colorectal cancer isn't specifically mentioned in the search results, the findings regarding the significance of CCAT2 in CRC advancement are probably relevant to this subtype because of the same molecular pathways implicated in CRC pathogenesis.

The significant overexpression of CCAT2, miR-17, and c-MYC could be used as a noninvasive and trustworthy investigation among Egyptian patients with IBD. This indicates that the lncRNAs are a promising tool for deciphering the molecular background of CRC. An improved prognosis and decreased incidence of colorectal cancer can be achieved with early detection.

5. Conclusion

In conclusion, CCAT2, miR-17 and c-MYC can be used as noninvasive biomarkers among CRC and IBD in Egyptian patients. In the UC group, the expression levels for CCAT2, miRNA-17, and c-MYC were upregulated compared to the control group. CCAT2 showed 98.9% sensitivity and 100% specificity, miRNA-17 showed 90% sensitivity and 100% specificity, and c-MYC showed 83.3% sensitivity and 93.3% specificity. For the CD group, the levels of CCAT2, miRNA-17, and c-MYC were likewise upregulated. CCAT2 exhibited 66.2% sensitivity and 93.3% specificity, miRNA-17 had 66.7% sensitivity and 100% specificity, while c-MYC showed 70% sensitivity and 100% specificity. In the CRC group, expression levels of CCAT2, miRNA-17, and c-MYC were

all upregulated. CCAT2 showed 86.7% sensitivity and 100% specificity; miRNA-17 showed 86.7% sensitivity and 99.8% specificity; and c-MYC showed 83.3% sensitivity and 100% specificity

6. Conflicts of interest

There are no conflicts to declare.

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