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MicroRNA-372 and Long Non-Coding RNA-HULC as Diagnostic Biomarkers of Colorectal Carcinoma in Egyptian Patients

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ABSTRACT

Background: Colorectal cancer (CRC) is a significant health issue albeit limited reports from Egypt. Long non-coding RNAs (lncRNAs) and circulating microRNAs (miRNAs/miRs) have been found to have dysregulated expression in CRC.

Objective: This study aimed to investigate the use of miR-372 and lncRNA-HULC as noninvasive diagnostic biomarkers for CRC as well as to study the interaction between their expression levels in CRC.

Methods: Using quantitative real-time PCR, the relative expression levels of miR-372 and lncRNA-HULC in serum were assessed in all study subjects (40 CRC patients and 30 healthy controls).

Results: When compared to controls, serum expression levels of miR-372 and lncRNA-HULC were found to be significantly higher among CRC patients. According to receiver operating characteristic (ROC) analysis, which is the most popular approach of reporting the diagnostic accuracy of dysregulated ncRNAs, miR-372 had an AUC (an area under the curve) value of 0.76, sensitivity of 74.2%, and specificity of 96.4% at 7.8 cutoff point. Concerning lncRNA-HULC in the current study, AUC was 0.71 with sensitivity of 71.50% and specificity of 83.7% at 8.35 cutoff point.

Conclusions: This study provided evidence that miR-372 and lncRNA-HULC are possible noninvasive diagnostic biomarkers for CRC. This work is the first that, as far as we know, to demonstrate the correlation between serum relative expression levels of miR-372 and lncRNA-HULC in CRC. Results of our study showed that high expression levels of miR-372 and lncRNA-HULC in serum could predict CRC and could distinguish CRC patients from healthy controls.

Keywords: CRC, Noncoding RNAs, MiRNA-372, LncRNA-HULC.

INTRODUCTION

One of the most common malignancies of the digestive system in the globe is colorectal cancer (CRC). The second-leading cause of death in America, after lung cancer, is colon cancer, which has the fourth highest incidence **(1).** Despite the paucity of data, it was discovered that CRC is significantly more common in Egypt **(2).**

The majority of colorectal cancers are regular type adenocarcinomas, which can be graded as either tumors that are weakly differentiated, moderately differentiated, or well-differentiated 1, 2, or 3. Histologically, it is further divided into low-grade (50–100% gland development for adenocarcinomas that are well-and moderately differentiated), and high-grade for those that are poorly differentiated (0-49% gland development) **(3).**

Since the mechanisms behind the formation of CRC are still unknown, understanding the molecular underpinnings of colon oncogenesis is vital **(1)**. The development of colorectal cancer can be caused by mutations in specific genes. Genes involved in repairing DNA pathways may exhibit such mutations **(4).**

There are several non-coding RNAs in the human transcriptome. Small noncoding RNAs (sncRNAs), which range in size from 18 to 200 nucleotides, and long noncoding RNAs (lncRNAs) with a length over 200 nucleotides, are two categories into which these non-coding RNAs are divided **(5).**

MicroRNAs are small, non-coding RNAs that have an average length of 22 nucleotides (miRNAs/miRs) **(6, 7).** Several studies have indicated that miRNAs are either tumour suppressors or oncogenes in human cancers **(8).** According to **Bartley *et al.*** **(9)** the miRNA was a potential ideal biomarker in CRC and 230 miRNAs were expressed differently in adenoma and CRC **(9).** Additionally, it was discovered that 31 miRNAs were either elevated or downregulated in the CRC cases' serum **(10).** Considering its diminutive size, stability, and resistance to RNase destruction, miRNA performs better as a molecular marker than mRNA **(6, 7).** Colorectal cancer (CRC) and other cancers have been shown to have dysregulated levels of microRNA-372 (miR-372); nevertheless, its specific function in cancer is yet unknown **(10- 12).**

LncRNAs control the stability & pattern of expression of the genome **(13, 14).**

A number of malignancies, including CRC, are prone to carcinogenesis due to nonfunctional lncRNAs **(1, 15).** The most increased lncRNA in hepatocellular carcinoma (HCC) and other cancers is highly

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upregulated in liver cancer (HULC) [16]. When HULC is silenced, carcinogenesis is reduced, while its over-expression is linked to increased tumor growth and a bad prognosis [17]. According to certain theories [18- 21], HULC might play an oncogenic role in colon cancer.

It's interesting to note that previous researches have linked between the miR-372 and higher levels of HULC expression in HCC [21- 24] and osteosarcoma [25]. Determining the use of miR-372 and lncRNA-HULC as non-invasive diagnostic biomarkers for CRC as well as researching the relationship between their expression levels in CRC patients are therefore the main goals of this research.

MATERIALS AND METHODS

Study participants: This case-control study included 30 Egyptian healthy controls with matched age and sex without any clinical signs of CRC or a family history of the disease and 40 Egyptian CRC patients who were chosen from Internal Medicine Department, Faculty of Medicine, Kasr Al Ainy Hospital, Cairo University. The age requirement for both groups was more than 18 years. Women who are pregnant, people with chronic infectious diseases, and people with a history of cancer and other illnesses were not included.

All study participants underwent the following: (1) A thorough medical history and general clinical examination. (2) Serum was obtained by centrifuging 5 ml of whole blood, which was then held for RNA extraction at -80 °C, reverse transcription into complementary DNA (cDNA), and measuring the relative expression levels of miRNA-372 & lncRNA-HULC in the serum using the quantitative real-time PCR (qRT-PCR).

Extraction of RNA: The guidelines provided by the maker were followed utilising the miRNeasy mini kit for extracting total RNA from serum (Qiagen, Germany, Cat. No. 74104). The NanoDrop®-1000 with the use of a spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), the purity of the RNA samples was assessed.

cDNA reverse transcription: Reverse transcription (RT) of extracted RNA was performed using RT2 first strand kit from Qiagen in Germany (Cat. No. 330404) to produce cDNA in accordance with the manufacturer's guidelines. The master mix for reverse transcription was made. Each tube containing the reverse-transcription master mix received a dose of template RNA. Gentle centrifugation used to mix the mixture. RT^2 first strand reverse transcriptase was inactivated using conventional PCR by incubating the extracted total RNA for 60 minutes at 37 °C and 5 minutes at 95 °C in a volume of 20 µL. Before proceeding, the cDNA of every sample was stored at -80 °C.

Quantitative real-time PCR: The Sybr Green/Master Mix RT^2 qPCR kit (Qiagen, Germany, Cat. No. 330520) and RT^2 qPCR primer assays for miR-372 and HULC were used (Qiagen, Germany, Cat. No. MS00004067 and 330701 respectively, GeneGlobe ID for HULC: LPH17802A-200). A short nucleolar RNA C/D box 68 (SNORD-68) was used to standardize the relative expression pattern of miR-372 as an endogenous housekeeping gene (Qiagen, Germany, Cat. No. MS00033712). Additionally, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Qiagen, Germany, Cat. No. 330701, GeneGlobe ID- QT00079247) was used as an internal control for lncRNA-HULC.

The Rotor-gene Q system thermocycler (Qiagen, USA) was used under specific settings, it comprised 45 cycles of each PCR step (denaturation, 95 °C for 15 s, and annealing/extension, 60 °C for 1 min) after holding stage at 95 °C for 10 min. Melt curve analysis was done to confirm the PCR reactions' specificity. The fractional cycle number at which the amount of amplified target hits a predetermined threshold is represented by the values of the Cycle Threshold (CT) which converted to an excel file and sent to the https://www.qiagen.com/dataanalysiscenter web page for RT2 PCR array data analysis.

Utilizing data from the website, using the $2^{-\Delta\Delta CT}$ technique for relative quantification, the fold change was computed. First, ΔCT was computed between the reference gene and the gene of interest on average. This was followed by ΔΔCT computations [ΔCT (patient)-ΔCT (control)]. The $2^{-\Delta\Delta CT}$ formula was then used to compute the fold change. Fold-regulation is equal to the fold change, and values of fold change greater than 1 imply positive or up-regulation. A fold change of less than one indicates a negative or down-regulation and fold regulation is the negative inverse of fold change. The control value was set to 1, which was assumed to be the normal value.

Ethics approval: Informed written consents were obtained from all individual participants included in the study. Research Ethics Committee (REC) at Cairo University's Faculty of Medicine examined and approved the current study in accordance with Declaration of Helsinki, (Ethics Approval Code: N-224-2023).

Statistical evaluation

For the data analysis, SPSS version 25 was used. Regarding the expression of biomarkers, the data were

reported as quantitative data using the mean, standard deviation (SD) and standard error (SE). Relative frequency (percentage) and frequency (count) were used to depict data with categories. To compare categorical data, the Chi square test was utilized.

In order to compare data from two groups, independent t-test was conducted, and to compare data among three groups or more, we used the one-way ANOVA test. The diagnostic potency of miR-372 and lncRNA-HULC were assessed utilizing the area under the curve (AUC) and receiver operating characteristic (ROC) analysis. AUC more than 0.5 was considered significant. For correlations between quantitative data, the Pearson correlation coefficient was employed. P values were regarded as statistically significant if they were less than 0.05.

RESULTS

The study comprised 40 CRC patients (mean age was 53.9 ± 11.7 years, 14 females and 26 males). Thirty healthy control subjects who were matched for age and sex made up the control group (mean age was 46.9 ± 9.6 years, 14 girls and 16 males). The mean age of CRC patients (n=40) was 53.9 years which was significantly higher than healthy controls (46.9 years, n=30).

The study subjects' demographic and laboratory characteristics:

Compared to the control group, the CRC group was significantly older (P=0.010) (table 1 and figure 1). Additionally, table (1) demonstrated that gender did not significantly different (P=0.33), while the CRC group had a majority of men accounting for 65.0% of the CRC population. The decreased hemoglobin in the CRC group also represents a CRC and control group difference that was statistically significant (P=0.04). Our findings indicated no statistically significant difference between the two groups' platelet counts, ALT levels, or AST levels.

Table (1): Demographic data of the studied groups

Variables		CRC (n=40)	Control (n=30)	p-value
Age (years)		53.9±11.7	46.9±9.6	**0.010** [a]
Gender	Female Male	14 (35%) 26 (65%)	14 (46.5%) 16 (53.5%)	0.33[b]
Hg (g/dL)		11±2.9	12.5±1.1	**0.04** [a]
PLT × 10^3/mm3		185.6±31.8	177±10.0	0.105 [c]
TLC × 10^3/mm3		6.79 ± 1.02	-	
ESR (mm/hr)		49.7±3.4	-	
Liver Function Tests				
ALT (IU/L)		21.9±1.1	22.2±4.07	0.779 [c]
AST (IU/L)		24.1±1.7	25.67±4.1	0.872 [c]
Bilirubin (mg/dL)		0.76±0.18	-	
Albumin (g/dL)		4.68±0.14	-	
Kidney Function Tests				
Creatinine (mg/dL)		0.91±0.25	-	
Tumor Markers				
CEA		30.31±4.7	-	
CA19.9		33.79±4.21	-	
AFP		9.94±6.83	-	

Values were expressed as mean ± SD and gender was presented by number (percentage). CRC, n=40, healthy controls, n=30. a One-way ANOVA, b Chi-squared test, c independent t-test. P values in bold are statistically significant (P < 0.05). CRC, colorectal cancer; Hg, hemoglobin; PLT, platelets count; ESR, erythrocyte sedimentation rate; TLC, total leukocyte count.; ALT, alanine transaminase; AST, Aspartate transaminase, CEA, Carcinoembryonic Antigen, CA, Cancer Antigen, AFP, Alpha-Fetoprotein.

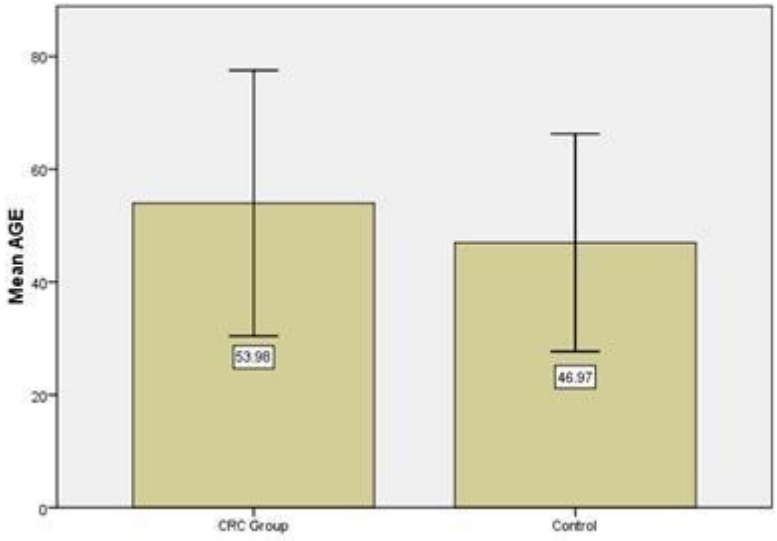

Figure (1): Mean age in different study groups. Values are expressed as mean.

The study CRC group's clinical and pathological characteristics:

Regarding clinical presentation, CRC patients displayed multiple symptoms at once. Constipation, weight loss, and stomach pain were seen in 22.5% of the patients. As demonstrated in table (2), 47.5% of the patients experienced both constipation and abdominal pain. According to table (2) and figure (2a), colonoscopy revealed that 80% of CRC tumors were in the colon, with the remaining 20% being rectal.

40% of CRC patients presented with a mass, 52.5% with ulcers, and the remaining 7.5% with hyperemia (Table 2 and figure 2d). In terms of histopathology, table (2) demonstrated that 90% of CRC group members had adenocarcinomas, while 7.5% of the group had mucinous CRC and the remaining were undifferentiated (Figure 2b).

Adenocarcinoma was further classified into well-differentiated (47.5%), moderately differentiated (32.5%) and poorly differentiated (10%) as demonstrated in figure (2c). Regarding CT picture, 15% of the CRC patients had both mass lesion and regional lymph node metastasis. While 10% of the patients had both mass lesion and liver metastasis as shown in table (2).

Table (2): Clinicopathological data of the CRC group.

Parameters	N (%)
Symptoms of presentation	
Abdominal pain	26
Constipation	29
Loss of weight	13
Bleeding per rectum	12
Anatomical site	
Rectum	8(20%)
Colon	32 (80%)
Tumor Grade	
Adenocarcinoma	36(90%)
Well-differentiated (grade I) (Low grade)	19 (47.5%)
Moderate-differentiated (grade II) (Low grade)	13 (32.5%)
Poorly-differentiated (grade III) (High grade)	4 (10%)
Undifferentiated carcinoma *(High grade)*	1(2.5%)
Mucious tumor *(High grade)*	3 (7.5%)
Colonoscopy picture	
Colonoscopy mass	16 (40%)
Colonoscopy ulcer	21 (52.5%)
Colonoscopy hyperemia	3 (7.5%)
CT Picture	
Mass lesion	22
Wall thickening	18
Regional lymph node (LN) metastasis	10
Liver metastasis	7

Values are expressed in numbers (percentage). CRC, n=40, healthy controls, n=30.

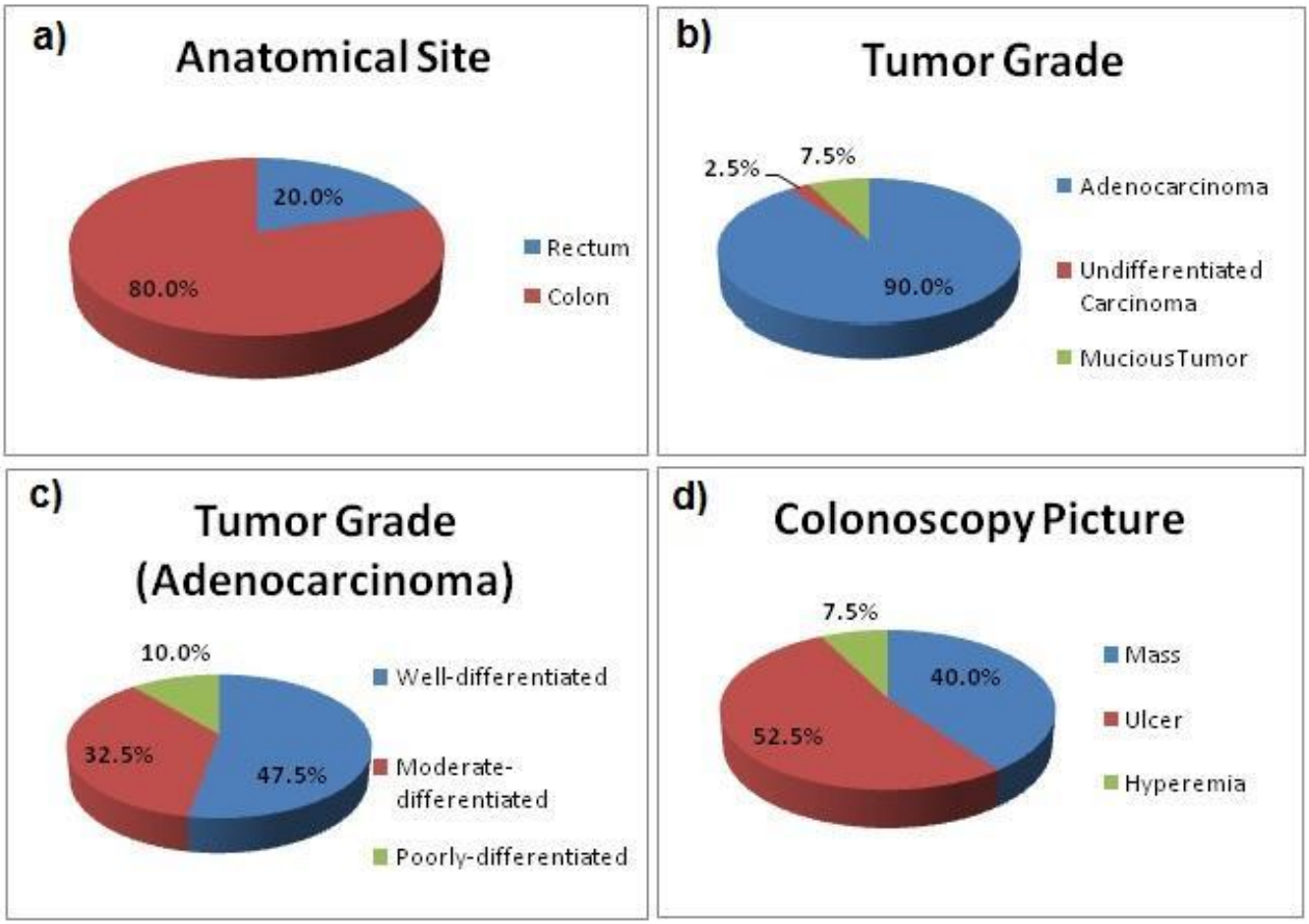

Figure (2): Distribution of anatomical site, tumor grade and colonoscopy picture in CRC patients (n=40). Data are expressed in numbers (percentage). **a)** Distribution of anatomical site in CRC patients (n=40): 80% of the CRC tumors were at the colon (n=32), while the remaining were rectal (n=8). **b)** Distribution of tumor grade in CRC patients (n=40): 90% of CRC group were adenocarcinoma (n=36), while 7.5% of CRC studied group were mucinous (n=3) and the remaining (2.5%) were undifferentiated (n=1). **c)** Distribution of adenocarcinoma subgroup in CRC patients (n=36): 47.5% of adenocarcinoma was well-differentiated (n=19), 32.5% was moderately differentiated (n=13) and 10% was poorly differentiated (n=4). **d)** Distribution of colonoscopy picture in CRC group (n=40): 40% of CRC patients were presented by mass (n=16), 52.5% were presented by ulcers (n=21), while the remaining (7.5%) were presented by hyperemia (n=3).

Relative expression levels of miR-372 and lncRNA-HULC: Table (3) demonstrated that serum samples from CRC patients were significantly overexpressed in terms of miR-372 and lncRNA-HULC relative expression levels when in comparison with the control group (p-values of 0.001 and 0.0001, respectively). Figure (3) illustrated the distribution of the fold change values for the investigated biomarkers.

Table (3): Serum relative expression levels of miR-372 and lncRNA-HULC in the study subjects.

Serum biomarkers	CRC	Control	p-value
MiR-372	7.47±1.56	0.98±0.010	**0.001[a]**
LncRNA-HULC	8.07±0.446	1.02±0.012	**0.0001[a]**

CRC, n=40, healthy controls, n=30. a One-way ANOVA. Standard error (SE) is used for expression of biomarkers. P values in bold are statistically significant (<0.05).

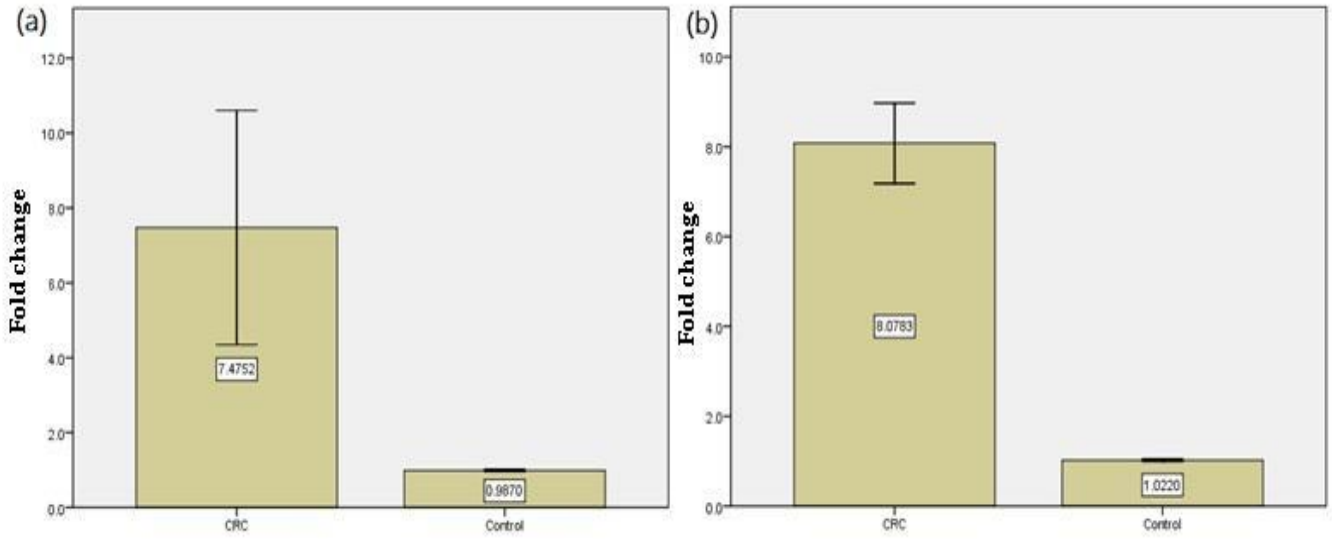

Figure (3): Serum relative expression levels of miR-372 and lncRNA-HULC among CRC patients (n=40) compared with healthy controls (n=30). (a) Fold change of serum miR-372 expression in patients with CRC compared to healthy controls, (b) Fold change of serum HULC expression in CRC patients compared to healthy controls. Data are expressed as mean.

Serum relative expression levels of miR-372 based on different variables among CRC patients: We discovered that, with regard to the colonoscopy image, patients without mass lesions had significantly higher expression levels of miR-372 than patients with mass lesions (p-value = 0.04). Additionally, hyperemic patients expressed miR-372 at significantly higher levels than those without hyperemia (p-value = 0.001). Patients without regional lymph node or liver metastasis exhibited significantly greater miR-372 expression levels when compared to patients with the condition (p-value = 0.04). Additionally, individuals with undifferentiated carcinoma had significantly higher serum relative expression levels of miR-372 than patients with poorly differentiated adenocarcinomas (p-value = 0.04). However, no other variables showed a statistically significant difference (Table 4).

Table (4): Serum relative expression levels of miR-372 based on different variables among CRC patients (n=40).

Parameters			**miR-372**	**p-value**
Anatomical Sites	Rectum		10.65±5.6	0.25
	Colon		6.67±1.40	
Colonoscopy Picture	Mass	Yes	5.2±1.4	**0.04**
		No	8.9±2.3	
	Ulcer	Yes	8.5±2.6	0.26
		No	6.3±1.5	
	Hyperemia	Yes	11.8±6.0	**0.001**
		No	7.1±1.6	
CT Picture	Mass lesion	Yes	6.6±2.3	0.58
		No	8.4±2.0	
	Wall thickening	Yes	8.3±2.0	0.59
		No	6.6±2.3	
	Regional LN metastasis	Yes	5.2±2.1	**0.04**
		No	8.2±1.9	
	Liver metastasis	Yes	5.6±2.8	**0.04**
		No	7.8±1.8	
Tumor Grades	Well-differentiated adenocarcinoma		8.20±3.09	**0.04** Poorly-differentiated adenocarcinoma versus undifferentiated carcinoma
	Moderate-differentiated adenocarcinoma		7.01±1.87	
	Poorly-differentiated adenocarcinoma		5.9±3.5	
	Undifferentiated carcinoma		11.6±0.1	
	Mucinous Tumor		6.5±2.6	

P values in bold are statistically significant (<0.05). Standard error (SE) is used for expression levels.

Serum relative expression levels of HULC based on different variables among CRC patients: Based on different variables as shown in table (5), there were no statistically significant variations in the lncRNA-HULC serum relative expression levels among CRC patients (P > 0.05).

Table (5): Serum relative expression levels of lncRNA-HULC based on different variables among CRC patients (n=40)

Parameters			lncRNA-HULC	p-value
Anatomical Sites	Rectum		7.76±0.62	0.31
	Colon		8.15±0.53	
Colonoscopy Picture	Mass	Yes	7.6±0.8	0.14
		No	8.3±0.5	
	Ulcer	Yes	8.5±0.5	0.13
		No	7.5±0.7	
	Hyperemia	Yes	6.7±1.6	0.39
		No	8.1±0.4	
CT Picture	Mass lesion	Yes	7.7±0.6	0.36
		No	8.5±0.6	
	Wall thickening	Yes	8.5±0.6	0.37
		No	7.8±0.6	
	Regional LN metastasis	Yes	7.0±0.9	0.51
		No	8.4±0.5	
	Liver metastasis	Yes	8.1±0.3	0.60
		No	8.0±0.5	
Tumor Grades	Well-differentiated		8.7±0.3	>0.05
	Moderate-differentiated		7.1±1.1	
	Poorly-differentiated		9.03±0.34	
	Undifferentiated carcinoma		6.6±0.1	
	Mucious Tumor		7.5±1.6	

P values in bold are statistically significant (<0.05). Standard error (SE) is used for expression levels.

Diagnostic performance of serum miR-372 and HULC:

To reflect the diagnostic accuracy of the results, AUC, sensitivity, and specificity of increased miR-372 and lncRNA-HULC were determined by utilizing ROC curves. A chosen cut-off is represented by each point on the ROC curve (Figure 4).

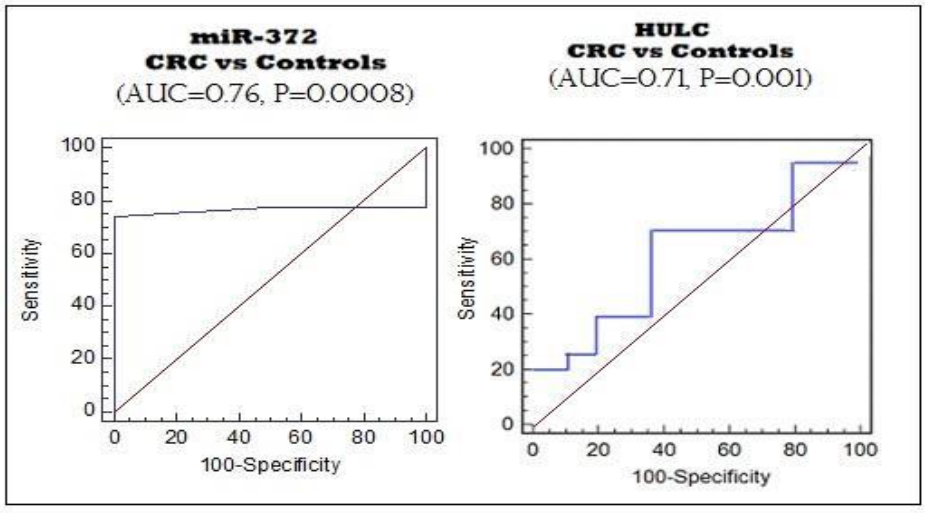

Figure (4): Diagnostic performance of serum miR-372 and HULC. ROC curve analysis of serum miR-372 and HULC to discriminate studied groups, CRC (n=40) and healthy controls (n=30)

According to ROC analysis, serum miR-372 distinguished CRC from healthy controls with an AUC of 0.76, a sensitivity and specificity of 74.2% and 96.4%, respectively, at a cutoff value of 7.8. (Table 6).

Table (6): The ROC curve analysis of serum relative expression levels of miR-372 for CRC diagnosis. P values in bold are statistically significant (P < 0.05). AUC, area under the curve

Serum biomarker	AUC	Cut-off	Sensitivity	Specificity	Accuracy	p-value
miR-372	0.76	7.8	74.2%	96.4%	85.3%	**0.0008**

P values in bold are statistically significant (P < 0.05). AUC, area under the curve.

While serum HULC distinguished CRC from healthy controls with an AUC of 0.71 and a sensitivity and specificity of 71.50% and 83.7%, respectively, at a cutoff value of 8.35 (Table 7).

Table (7): The ROC curve analysis of serum relative expression levels of lncRNA-HULC for CRC diagnosis

Serum biomarker	AUC	Cut-off	Sensitivity	Specificity	Accuracy	p-value
LncRNA-HULC	0.71	8.35	71.50%	83.7%	77.6%	**0.001**

P values in bold are statistically significant (P < 0.05). AUC, area under the curve.

Correlation of serum relative expression levels of miR-372 and lncRNA-HULC with demographic and laboratory parameters in CRC patients: MiR-372 and PLT have a negative correlation as indicated in table (8) and figure (5) (P-value=0.04, correlation coefficient (r) = -0.301). In CRC patients, there was no statistically significant correlation between miR-372 and any other demographic or laboratory data. Similar to this, no significant correlation between lncRNA-HULC and any demographic or laboratory variable was discovered in CRC patients.

Table (8): Correlation of serum relative expression levels of miR-372 and lncRNA-HULC with demographic and laboratory parameters in CRC patients (n=40).

Parameters	miR-372 (r)	p-value	lncRNA-HULC (r)	p-value
Age	-0.093	0.56	-0.092	0.57
Gender	-0.229	0.15	0.148	0.36
Hg	0.151	0.35	0.122	0.45
PLT	-0.301	**0.04**	0.229	0.15
ALT	-0.199	0.21	0.243	0.13
AST	0.072	0.65	0.214	0.18
TLC × 103/mm3	0.76	0.64	0.17	0.27
ESR (mm/hr)	-0.127	0.46	-0.09	0.58
Bilirubin (mg/dL)	0.075	0.64	0.071	0.066
Albumin (g/dL)	0.020	0.211	0.080	0.62
Creatinine (mg/dL)	0.28	0.07	0.13	0.41
CEA	0.105	0.52	0.137	0.407
CA19.9	0.045	0.78	0.23	0.14
AFP	-0.081	0.61	-0.019	0.907

(r), Pearson correlation coefficient; p-values in bold are statistically significant (P < 0.05)

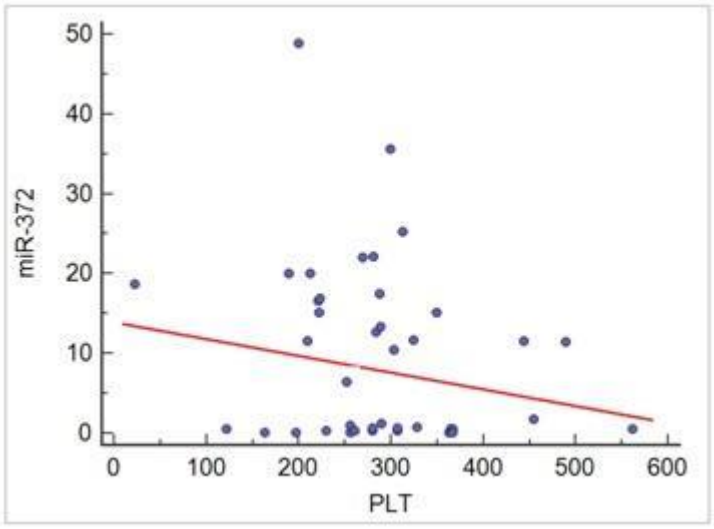

Figure (5): Correlation between miR-372 and PLT among CRC patients (n=40). Negative correlation between miR-372 and PLT (P-value=0.04, correlation coefficient (r) =-0.301). PLT, platelets count.

DISCUSSION

One of the most prevalent malignancies, CRC has a high mortality rate, a hidden incidence, and rapid progression. Clarifying the molecular pathways of colon oncogenesis is essential because the precise mechanisms of CRC development are still unknown [(2)].

According to previous reports, miR-372 may play either an oncogenic or a suppressive role in numerous human malignancies, including CRC [(11, 12)]. Emerging research has suggested that lncRNAs play critical roles in the development and progression of colon cancer, with the discovery of 200 lncRNAs that were differently expressed in colon tumors using RNA sequencing data from the TCGA dataset [(26)]. These findings imply that lncRNA-HULC and miR-372 play a crucial role in colon carcinogenesis, which is what drives us to investigate their possible role as diagnostic biomarkers in CRC.

The mean age of our CRC patients was 53.9 years, which was significantly older than the mean age of the healthy controls (46.9 years) and this could be attributed to the natural history of acquired diseases in older people, which is higher than in younger people. Our findings are consistent with those of **Phiphatpatthamaamphan and Vilaichone** [(27)] who previously reported comparable findings.

Hemoglobin levels were statistically significantly lower in the CRC group of this study compared to the control group. This is in line with a prior study that found cancer-related anemia to be a frequent complication in almost all malignant illnesses [(28)].

The current study's findings demonstrated that when compared to the control group, blood samples from CRC patients had significantly higher relative expression levels of miR-372 and lncRNA-HULC (p-values = 0.001 and 0.0001, respectively). This finding is consistent with previous research that found blood or tissue levels of miR-372 were considerably elevated in CRC patients as compared to healthy controls or normal tissues [(11, 29)]. Additionally, miR-372 was one of the 31 miRNAs that **Carter *et al.*** [(30)] found to be dysregulated in CRC cases in a meta-analysis research. In CRC cells, **Wang *et al.*** [(11)] found that a number of genes were new miR-372 targets. Additionally, their research showed that miR-372 is a major contributor to the interaction between the Wnt/b-catenin and NFkB pathways.

In the current study, patients without regional lymph node or liver metastases had significantly higher serum relative expression levels of miR-372 (p-value = 0.04) compared to groups that had these conditions. This finding might be explained by the current study's relatively small sample size of CRC patients who had liver or regional lymph node metastases. Additionally, the present study found that patients with hyperemia and no mass lesions had significantly greater levels of miR-372 expression than patients with lesions and those without hyperemia (p-values, respectively, 0.04 and 0.001). We discovered that miR-372 levels were substantially higher in patients with undifferentiated carcinoma than those with poorly differentiated adenocarcinoma (p-value = 0.04). However, other variables did not show any statistically significant differences. This result could be explained by the small number of samples that were examined.

Overexpression of serum HULC levels in CRC patients in the current study are consistent with previous studies that identified HULC as an oncogenic long noncoding RNA in CRC. It's interesting to note that

HCC and colorectal carcinomas with liver metastases have both been associated with HULC expression [31].

According to **Yang and his colleagues** [32], CRC cell lines and tissues had increased levels of HULC expression**.** Additionally, a prior investigation on CRC patients from Egypt found that when CRC patients' serum HULC was compared to healthy controls, it was significantly higher [33].

In the current study, no statistically significant difference was identified in the serum relative expression levels of lncRNA-HULC based on various variables among CRC patients. This result could be attributed to the small number of samples that were tested. To our knowledge, there is no prior research has examined the relationship between miR-372 and HULC expression levels in relation to CRC. However, several prior studies have demonstrated the significance of this association in carcinogenesis [25, 34, 35].

Using ROC curves, the most widely used method of reporting the diagnostic accuracy of dysregulated ncRNAs [30], miR-372 has an AUC value of 0.76, sensitivity of 74.2%, and specificity of 96.4% at 7.8 cutoff point. This finding is consistent with **Yu *et al.*** [29] findings which showed that high levels of miR-372 expression in serum or tissue might strongly predict CRC. Concerning lncRNA-HULC in the current study, the ROC curve's AUC was 0.71 with 71.50% sensitivity and 83.7% specificity at 8.35 cutoff point. Similar to our results, serum HULC was found to be used by an earlier study on CRC patients in Egypt to distinguish CRC from healthy controls using ROC analysis [33].

Regarding the relationship between our two understudied biomarkers (miR-372 and lncRNA-HULC) and the laboratory results of CRC patients, it was discovered that there was a significant inverse relationship (P-value=0.04, correlation coefficient (r) = -0.301) between the level of miR-372 and PLT. The level of miR-372 and PLT in HCC, on the other hand, were found to be significantly positively correlated in a prior study [35].

CONCLUSION

Our data showed that miR372 and lncRNA-HULC may have a role in the genetic predisposition to CRC. However, more research on the molecular mechanisms, targets, and related signaling pathways of these two biomarkers in the development of cancer is required, as well as bigger sample sizes.

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ABBREVIATIONS

AFP: alpha-Fetoprotein, **ALT:** alanine transaminase, **AST:** aspartate transaminase, **AUC:** area under the curve, **CA:** cancer antigen, **CEA:** carcinoembryonic antigen. **CRC:** colorectal cancer **ESR:** erythrocyte sedimentation rate **HCC:** hepatocellular carcinoma **Hg:** hemoglobin, **HULC:** highly upregulated in liver cancer **LncRNAs:** long non-coding RNAs **MiRNAs/MiRs:** microRNAs, **PLT:** platelets count, **qRT-PCR:** quantitative real-time polymerase chain reaction **ROC:** receiver operating characteristic **SD:** standard deviation**SE:** standard error, **TLC:** total leukocyte count

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