International Journal of Biomedicine 14(4) (2024) 575-582
http://dx.doi.org/10.21103/Article14(4)_OA6

ORIGINAL ARTICLE

Oncology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Expression of Long Non-Coding RNA H19 and miR-675 in Patients with Colorectal Cancer and Ulcerative Colitis

Rania Sobhy Abu Taleb[1], Olfat Shaker[2], Ahmed Khairy[3], Dalia A. Gaber[1,4*]

[1]*Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Helwan University, Cairo, Egypt*
[2]*Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Cairo University, Egypt*
[3]*Endemic Medicine Department, Faculty of Medicine Kasr Al-Ainy, Cairo University, Egypt*
[4]*College of Medicine, Gulf Medical University, Ajman, United Arab Emirates*

Abstract

Background: Colorectal cancer (CRC), which has a high mortality rate, is one of the most common malignancies. It is a growing public health problem that recently encompasses the younger age group. Inflammatory bowel disease (IBD), like ulcerative colitis (UC), is a risk factor for CRC. The aim of this study was to test the expression of microRNA-675 (miR-675) and long noncoding RNA H19 (H19) for their diagnostic and prognostic potential in CRC and UC.

Methods and Results: This prospective cross-sectional study included 60 patients diagnosed with CRC, 60 patients with UC, and 30 healthy subjects as controls. The real-time RT-PCR technique quantified the expressions of the non-coding RNAs (ncRNAs) miR-675 and lncH19. The biochemical and radiological findings were assessed and correlated with the expression of the genetic biomarkers.

H19 showed high expression in both CRC and UC groups, which is significantly different from the control group. There was a significantly enhanced expression of H19 in CRC, compared to the UC group. Increased H19 expression was detected in the caecum and ascending colonic lesions, with a significant difference from flexures and transverse colon (P=0.010), and recto-sigmoid colon (P=0.05). Although miR-675 showed downregulation in both UC and CRC groups, it revealed a higher expression in CRC when compared to the UC group.

Conclusion: Based on our findings, we can conclude that H19 can be a potential noninvasive biomarker for the diagnosis of UC and early detection of CRC.**(International Journal of Biomedicine. 2024;14(4):575-582.)**

Keywords: lncH19 • miR-675 • colorectal cancer • ulcerative colitis

For citation: Abu Taleb RS, Shaker O, Khairy A, Gaber DA. Expression of Long Non-Coding RNA H19 and miR-675 in Patients with Colorectal Cancer and Ulcerative Colitis.. International Journal of Biomedicine. 2024;14(4):575-582. doi:10.21103/Article14(4)_OA6

Abbreviations

CA19-9, carbohydrate antigen 19-9; **CEA**, carcinoembryonic antigen; **CRC**, colorectal cancer; **IBD**, inflammatory bowel disease; **H19**, long non-coding RNA (lncRNA) H19; **miRNA**, microRNA; **miR-675**, miRNA-675; **ncRNAs**, non-coding RNAs; **PCR**, polymerase chain reaction; **SNORD68**, small nucleolar RNA, C/D box 68; **UC**, ulcerative colitis.

Introduction

Colorectal cancer (CRC) is among the fatal types of cancer worldwide.[1] It represents 8.5% of the total cancer mortalities. Lung and liver metastasis are the main causes of mortality.[2] The probability of having CRC is about 4%–5%. Associated risk factors comprise age, lifestyle, and chronic inflammatory bowel disease (IBD). Colorectal carcinomas are classified as sporadic (70%), inherited (5%), and familial (25%).[3] Most CRCs (75%-80%) are diagnosed at stages I-III,

while 20%–25% of patients are diagnosed at stage IV. This patient subgroup has the worst outcome, with a 5-year survival of around 10%.[4]

Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are cancer antigens used as biomarkers for CRC. However, they tend to elevate during the late course of the disease with significantly increased serum levels in metastases.[5]

In Egypt, IBD prevalence is 11.2%, with UC being predominant over Crohn's disease with a ratio of 6:1.[6] The incidence of IBD is increasing worldwide. Globally, IBD will affect up to 30 million individuals by 2025.[7]

For patients with long-standing colonic inflammation, CRC is an expected complication.[8] Detecting dysplasia with endoscopy is difficult and can make early diagnosis quite challenging.[9]

Exploring reliable and non-invasive biomarkers (lncRNAs, miRNAs) for UC and CRC allows a detailed understanding of the molecular mechanisms underlying carcinogenesis. The possibility of relying on such biomarkers also provides early detection, rapid intervention, and, thus, prevention of complications.

MicroRNAs (miRNAs) are epigenetic regulatory molecules that downregulate gene expression by complementarily binding to the target mRNA.[10] MicroRNAs are involved in gastrointestinal pathologies by targeting the transcripts encoding proteins of the intestinal barrier and their regulators, which are associated with inflammation and colon cancer.[11]

Previous research revealed that miR-675 is derived from exon 1 of long non-coding RNA (lncRNA) H19 (H19). H19 has a tumor-suppressing activity, and its derivative, miR-675, has been shown to be oncogenic in gastric,[12] liver,[13] and lung cancer.[14] Hence, miR-675 is dysregulated in various types of cancers and may serve as a potential detector of carcinogenesis.

H19 is known to express in primary CRC tissues and is further upregulated in metastatic lesions, as proved by database analysis and investigation of clinical specimens. It targets the heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), activates Raf-ERK signaling, and induces epithelial-mesenchymal transition, resulting in the metastases of CRC cells. Hence, H19 could be a potential prognosis biomarker and therapeutic target for CRC.[15]

In our presented study, we examined the possibility of relying on H19 and miR-675 as genetic detectors of UC and markers for diagnosing CRC.

Materials and Methods

Study Design

This prospective cross-sectional study included 60 patients diagnosed with CRC, 60 patients with UC, and 30 healthy subjects as controls.

Inclusion criteria: patients above 18 years, the gender of both sexes, CRC patients with any stage (confirmed by histopathology), and UC Patients (confirmed by histopathology).

Exclusion criteria: any cancer other than CRC and patients who receive chemotherapy or radiation.

Each participant underwent a comprehensive examination, including history taking, clinical examination, laboratory and radiological examination, and the assessment of H-19 and miR-675 expression.

From each participant 5 ml of blood was drawn using sterile labeled vacutainers. Samples were centrifuged at 3000g for 10 min; sera were stored at -80°C till RNA extraction.

Total RNA Extraction and Reverse Transcription

Total RNA was extracted using miRNeasy mini kit and protocol for purification of total RNA, including miRNA (Qiagen, Germany. Cat. No.217004). Extracted RNA was subjected to RNA quantitation and purity assessment using NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA). Reverse transcription (RT) was carried out using the miScript® II RT kit and protocol for converting RNA-RT into cDNA (Qiagen, Germany. Cat. No. 218161) as a part of the miScript PCR system, which uses total RNA that contains ncRNA as the starting material for cDNA synthesis.

Detection of lncRNA H19 and mature miR-675

PCR primers

Target-specific Primers Assay for lncRNA H19 and miR-675 were supplied by Qiagen, Germany. SNORD68 and GAPDH were used as endogenous housekeeping genes. qRT-PCR was carried out using a miScript SYBR® Green PCR kit and protocol for miRNA and lncRNA quantitative detection (Qiagen, Germany. Cat. No. 218073). All samples were analyzed using the Rotor gene Q RT-PCR System (Qiagen, Valencia, CA, USA). The $2^{-\Delta\Delta Ct}$ method was conducted for the analysis and measurement of relative gene expression levels.[16]

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS version 17.0 (Chicago: SPSS Inc.). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean ± standard deviation (SD) or mean ± standard error of the mean (SEM) for continuous variables. Differences in continuous variables between the two groups were calculated using the independent Student's t-test. Multiple comparisons were performed with one-way ANOVA followed by the Bonferroni test. Categorical variables were analyzed using the chi-square test. Receiver operating characteristic (ROC) curves were used to evaluate the performance of diagnostic tests. A P-value of ≤ 0.05 was considered statistically significant.[17]

Results

Concerning age, the population of the CRC group was significantly older than that of the UC group (P=0.0001). No statistically significant difference was noted between UC and the control group. No statistically significant difference was found between groups concerning gender (Table 1).

Expression of miR-675 and lncRNA H19 in CRC and UC

Gene expression analysis of miR-675 revealed that it was under-expressed in both groups. However, its expression was significantly higher in CRC than in the UC group (P=0.001)

(Figure 1a). H19 showed an enhanced expression in the CRC group, which was significantly different from the UC group and the control group (P=0.0001) (Figure 1b).

Table 1.

Demographic data of the study groups.

Variable	CRC (n=60) [a]	UC (n=60) [b]	Control (n=30) [c]	*P*-value
Age, years (M±SE)	53.07±1.96	32.10±1.99	31.06±1.04	P_{a-c}=0.0001 P_{b-c}= 0.644 P_{a-b}= 0.0001
Gender Female Male	 20 (33.3%) 40 (66.7%)	 24 (40%) 36 (60%)	 14 (46.6%) 16 (53.4%)	P_{a-c}=0.079 P_{b-c}=0.309 P_{a-b}=0.305

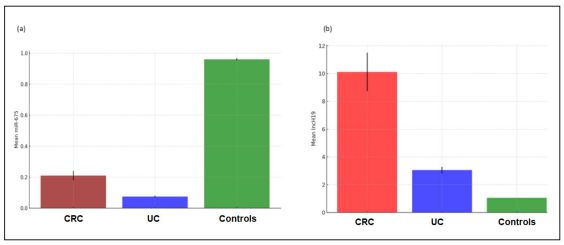

Fig. 1. *Gene expression in the study groups*

(a) The mean miR-675 expression levels were 0.2±0.03, 0.07±0.007, and 0.96±0.008 in CRC, UC, and controls, respectively.
(b) The mean lncH19 expression levels were 10.12±1.39, 3.05±0.24, and 1.05±0.008 in CRC, UC, and controls, respectively.

Laboratory and histopathological findings and lncH19 expression in the CRC group

Anemia was noted in CRC patients, as evidenced by decreased mean hemoglobin concentration, with a marginally significant difference compared to the control. There was a significant difference in platelet count, which was increased in CRC cases compared to the control (Table 2). Regarding the other laboratory investigations, no significant difference was found between the CRC and control groups. No correlation was detected between the level of H19 expression and any of the measured laboratory parameters.

Table 2.

CRC group: Laboratory findings.

Variable	CRC (n=60) mean±SD	Control (n=30) mean±SD	*P*-value
Hb (g/dL)	10.85±2.94	12.50±1.1	0.051
Platelets	269.17±131.827	177±100.0	0.010
ALT (0-42 IU/L)	21.925±10.07	22.2±9.07	0.779
AST (0-42 IU/L)	24.125±10.69	25.67±8.1	0.872
Bilirubin (mg/dL)	0.74±0.34	0.88±0.15	0.494
Albumin (3.5-5.5 g/dL)	4.43±0.94	4.73±0.4	0.6
AFP,n (mg/dL)	9.93±6.83	-	-

Hb, hemoglobin, ALT, alanine transaminase, AST, aspartate transaminase, AFP, alpha-fetoprotein.

About 28.3% of the malignant lesions were recto-sigmoid. Increased H19 expression was detected in the caecum and ascending colonic lesions, with a significant difference from flexures and transverse colon (P=0.010), and recto-sigmoid colon (P=0.05) (Figure 2).

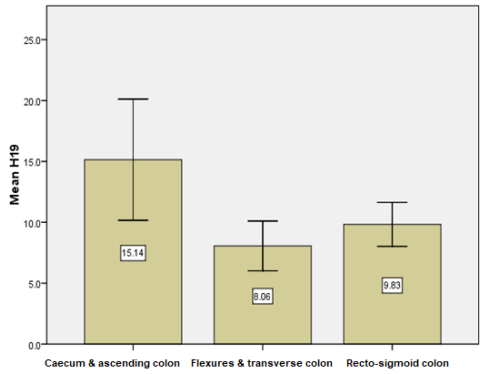

Fig. 2. *H19 expression according to CRC anatomical site.*

CT findings included mass lesion (38.3%), wall thickening (20%), regional lymph nodes (23.3%), and liver metastasis (11.7%). There was no significant correlation between H19 expression and the CT findings. Regarding the histopathological grades, 38.3% of patients were categorized as adenocarcinoma Grade I, 51.7% as adenocarcinoma Grade II, and 10% as mucinous tumor Grade II. No statistically significant difference in H19 expression levels concerning histopathological types or grades existed.

Laboratory findings, severity and extent of the disease, and lncH19 expression in the UC group

There was a significant decrease in Hb and hematocrit values and albumin level in the UC group compared to the control group (P=0.005, P=0.029, and P=0.001, respectively). CRP and ESR were markedly elevated in UC patients, with significant differences compared to control cases (P=0.0001). Total leukocyte count and levels of neutrophils and platelets were also elevated in UC patients, with significant differences compared to control cases (P=0.030, P=0.001, and P=0.042, respectively) (Table 3).

Table 3.

UC group: Laboratory findings.

Variable	UC (n=60) mean ± SEM	Control (n=30) mean ± SEM	*P*-value
Hb	11.36±0.33	12.48±0.24	0.005
Hematocrit	34.78±1.04	39.10±0.62	0.029
Total leukocyte count	8.61±0.82	6.23±0.25	0.030
Neutrophils	54.02±2.82	48.72±1.14	0.001
Platelets	315.68±18.28	296.67±11.29	0.042
CRP	15.85±3.53	1.99±0.22	0.0001
ESR	30.75±4.48	7.23±0.30	0.0001
Albumin	3.79±0.14	4.58±0.049	0.001

There was a significant increase in H19 expression in the group with moderate/severe UC compared to cases with remission (P=0.047) and mild cases (P=0.048) (Figure 3a). H19 showed the highest expression in left-sided lesions, which was significantly different from pancolitis lesions (P=0.05) (Figure 3b).

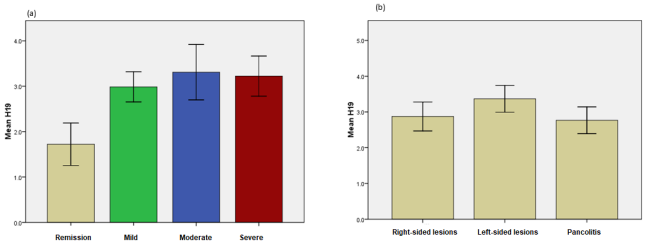

Fig. 3. *Pattern of H19 expression according to the severity and extent of UC.*

(a) lncH19 expression and the UC severity.

(b) lncH19 expression and the UC extent.

Diagnostic potential of H19 in CRC and UC

ROC curve analysis showed that H19 could be used in the diagnosis of UC, with a sensitivity of 91.7%, a specificity of 99.3% (AUC: 0.917, 95% CI: 0.852-0.959, P<0.0001) at a cut-off value of 2.659, and an accuracy of 95.5%.

ROC analysis revealed that H19 could discriminate between CRC and control subjects with a sensitivity of 90.0% and a specificity of 99.8% (AUC: 0.90, 95% CI:0.7919 -0.9667, P<0.0001) at a cut-off value of 8.73, and an accuracy of 96.85%.

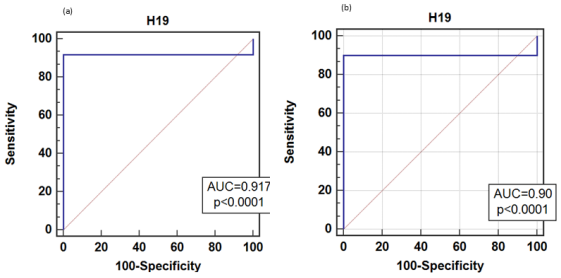

Fig. 4. *ROC curve analysis for H19 expression.*

(a) Diagnostic performance of H19 for UC, best cut-off value = 2.659 [sensitivity=91.7%, specificity=99.3%, and accuracy 95.5%], AUC=0.917, 95% CI:0.852-0.959, P<0.0001.

(b) Diagnostic performance of H19 for CRC, best cut off-value = 8.73 [sensitivity= 90.0%, specificity=99.8 %, and accuracy 96.85%], AUC=0.90, 95% CI:0.7919-0.9667, P<0.0001.

Discussion

The annual incidence of CRC is 1.4 million cases, resulting in 694,000 deaths.[18] The initiation of CRC is a complex biological process that involves multiple genomic and epigenomic alterations. The escalating incidence and the unpleasant outcome of CRC have drawn scientific attention and efforts toward continuous trials aiming to discover the underlying pathological processes of CRC and arrest these processes and, thus, CRC progression.[19]

Ulcerative colitis is an inflammatory bowel disease with a high incidence in developed countries and an accelerating incidence in developing countries. It results in severe morbidity, substantial healthcare costs, and loss of productivity at work.[20] This chronic colonic inflammation may precipitate CRC.[8] Analysis of CRC risk in patients with IBD showed a risk of 2% after 10 years of UC onset, 8% after 20 years, and 18% after 30 years after the onset of colitis.[9]

Hence, searching for early, non-invasive diagnostic biomarkers is required for early prediction and avoidance of such deleterious complications.[21] The diagnostic accuracy of CEA is considered low, with a sensitivity of 50% and a specificity of 80%.[22]

For the past decade, research has been directed to exploring the potential of using ncRNAs for diagnosis and prognosis of cancerous and pre-cancerous conditions. The current study aimed to test the expression of miR-675 and lncRNA H19 for their diagnostic and prognostic potential in CRC.

Our study showed a significant difference in age between the studied groups. The CRC group showed a statistical significantly higher mean age than the control and UC groups. This agrees with other studies that have found that CRC is mainly a disease of people over 55.[3,10,23,24] These results were previously challenged in an Egyptian study that recognized a relatively high percentage of patients diagnosed with CRC at a younger age group (between 20 and 40 years).[25]

Regarding the mean age in the UC group, it was recognized in previous work by Kathpalia et al.[26] that IBD has a bimodal peak of incidence, one in the second decade, which matches our patients' demographic data, and another one after the fifth decade, which is alleged to have a high mortality rate.

A study conducted by Rubin et al, in 2019 [8] mentioned that the peak age for UC is from 15 to 30 years, while another study[27] declared that the peak age of disease onset is between 30 and 40 years and that disease onset after 60 years tends to be a milder presentation.

Gender did not vary significantly among groups, although the male gender was slightly predominant in all study groups. The increase in males was higher in the CRC group than in the UC group. A study by Kim et al.[28] explained this by finding that social and cultural barriers could delay diagnosis in females.

miRNAs are responsible for the negative modulation of up to 60% of protein-coding genes.[29] miRNA expression is related to the increase of tumor mass size, metastasis, and increased malignancy grade of tumor cells. Several reports have demonstrated that miRNAs can be potential biomarkers for the diagnosis and prognosis of CRC.[30]

The dysregulated non-coding miRNA-675 may be used as a potential biomarker for detecting carcinogenesis in multiple types of cancer. It promotes CRC cell proliferation through its negative regulation of tumor suppressor DMTF1.[29]

In our study, miR-675 was under-expressed in both groups. However, expression was significantly higher in the CRC than in the UC group. Downregulation of miR-675 may result from reduced conversion of H19 into pre-miR-675 and/or reduced conversion of pre-miR-675 into mature miR-675,[11] for which the underlying cause needs to be elucidated.

In a study by Ren et al.,[31] miR-675 was upregulated in gastric cancer with overexpression of H19, promoting cell proliferation and inhibiting cell apoptosis.

In our work, H19 was highly expressed in both the UC and CRC groups. Expression was significantly higher in the CRC group.

Analysis of the public database showed that H19 is one of the most overexpressed lncRNAs in primary tumors and metastatic tissues when compared with its expression levels in the adjacent normal tissues in colorectal cancer.[32,33] These findings align with our current study.

The expression level of H19 was correlated with tumor differentiation and advanced TNM cancer stage in a study by Han et al.[34] In a study by Ismail et al.,[34] the expression level of H19 in Egyptian CRC patients showed an 11.38-fold increase compared to the controls.

lncRNA H19 and miR-675 play crucial roles in metastasis through the regulation of critical events, specifically the epithelial-to-mesenchymal (EMT) and mesenchymal-to-epithelial (MET) transitions.[35]

Ma et al.[36] investigated the expression of miR-675 in human pancreatic ductal adenocarcinoma (PDAC) tumors and cells, the biological function of miR-675 in PDAC cell proliferation, and the possible relationship among H19, miR-675, and E2F-1. As a transcript of the first exon of H19, the level of miR-675 was negatively correlated with H19 expression in microdissected PDAC tissues (r=-0.0646, P=0.001). The authors concluded that there might be a H19/miR-675/E2F-1 regulatory loop in cell cycle modulation.

The role of H19 in the pathogenesis of UC was mentioned in previous studies, which stated that the expression level of H19 was significantly higher in UC tissues than in paired normal tissues.

Zou et al.[37] reported that H19 overexpression inhibits expression of the TJ protein zonula occludens 1 (ZO-1) and the AJ protein E-cadherin at the post-transcriptional level via release of miR-675 embedded in H19 exon 1, and disrupts epithelial barrier function in an in vitro model using cultured intestinal epithelial cells.

A study by Lin et al.[38] found that intestinal H19 was dramatically upregulated in mice colitis models, as well as in inflamed colonic tissues from patients with IBD and that highly expressed H19 repressed the function of mRNAs encoding TJ protein ZO-1 and AJ protein E-cadherin by releasing miR-675, leading to epithelial barrier damage.

Regarding laboratory studies in our work, anemia was noted in CRC patients as evidenced by decreased mean hemoglobin concentration, with a marginally significant difference compared to control. This comes in agreement with Schneider et al.[39] There was also a significant increase in platelet count in the CRC cases compared to controls. In a study by Zhu et al.,[40] platelet indices were investigated as diagnostic markers for CRC. Platelet count was found to be elevated compared to patients with colon adenoma. No correlation was detected between the H19 expression level and any of the measured laboratory parameters in our study.

Recto-sigmoid lesions represented the major site of lesions in our study. The highest expression of H19 was found in the caecum and ascending colonic lesions, with a significant difference from flexures and transverse colon, and recto-sigmoid colon.

Chen et al.[41] found that overexpression of H19 was identified to be associated with pre-treatment metastasis, poor differentiation level, and advanced TNM stages.

H19 expression did not vary significantly with any CT findings in CRC, including mass lesion, wall thickening, regional lymph node involvement, or liver metastasis. Also, no statistically significant difference was found between H19 expression level and histopathological types or grades.

Laboratory investigations of UC patients showed anemia, manifested by low hemoglobin and hematocrit values, elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Chronic diarrhea affects iron absorption from the intestine; thus, anemia occurs.[42] In a study by A. Ananthakrishnan,[43] an elevated CRP or ESR was associated with an increased risk of CRC in UC patients. Serum albumin was relatively lower in UC and varied significantly compared to the control. The main cause of hypoalbuminemia in UC patients is the malabsorption of food protein constituents.

The severity of UC in our group of patients was categorized as mild in more than one-third of cases, while the other two-thirds were divided between moderate and severe, while only two patients were on remission based upon Mayo score (0-10). The score was classified into four different activities: Remission (0-2), Mild (3-5), Moderate (6-10) and Severe (>10). There was a significant increase in H19 expression in the group with moderate/severe UC compared to cases with remission and mild cases.

According to colonoscopy in our studied group, most cases were either left-sided colitis or pancolitis, and a small percentage were diagnosed with proctitis only. H19 showed the highest expression in left-sided lesions, which was significant from pancolitis lesions (P=0.05). ROC curve analysis showed that H19 could help in the diagnosis of UC, with a sensitivity of 91.7%, a specificity of 99.3% (AUC: 0.917, 95% CI: 0.852-0.959, P<0.0001) at a cut-off value of 2.659, and an accuracy of 95.5%.

A comparative analysis between H19 and CRP was conducted to assess their diagnostic value in UC in a study by Wang et al.[44] It was found that H19 and CRP levels increase with the deterioration of UC, with superior performance of H19. At the cut-off value of 2.755, H19 showed a high conformance to the clinical diagnosis of UC.

In a study by Shaker et al.,[45] H19 and miRNA-675-5p showed enhanced expression in UC and Chron's disease (CD) patients with marked significance from controls, especially in UC cases. Additionally, ROC analysis revealed that H19

could discriminate between UC and control subjects with a sensitivity of 94.3% and a specificity of 90.0% and between CD and control subjects with a sensitivity of 87.5% and a specificity of 88.5%. Furthermore, miR-675-5p was able to discriminate between UC and control subjects with a sensitivity of 85.7% and a specificity of 97.3% and between CD and control subjects with a sensitivity of 88.4% and specificity of 95.2%. Also, H19 and miRNA-675-5p showed enhanced expression in UC and Chron's disease patients with marked significance from controls, especially in UC cases.

Conclusion

Our study assessed the expression of H19 and miR-675 in Egyptian patients with CRC and UC. H19 was markedly expressed in CRC patients compared to UC. H19 can be considered a potential noninvasive diagnostic biomarker for CRC, with a sensitivity of 90.0% and a specificity of 99.8%. H19 could discriminate between UC and control subjects with a sensitivity of 91.7% and a specificity of 93.3%. Although miR-675 showed downregulation in both UC and CRC groups, it revealed a higher expression in CRC when compared to the UC group.

Further studies should be conducted on larger numbers of candidates. Studies should investigate the cause of sequestration of miR-675 in ulcerative colitis and colorectal cancer, though it is formed from its precursor H19. Other miRNAs should be used to make the miRNAs' signature through the development of panels containing many miRNA biomarkers and lncRNA. Conventional carcinoembryonic antigen (CEA) can be added to these panels in the diagnosis and screening of ulcerative colitis and colorectal cancer, which helps achieve more accuracy.

Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of Affiliated Teaching Hospital of Kasr AL-Aini, Cairo University, Egypt [code: MS-41-2020]. The study was conducted in accordance with the ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013). All participants provided written informed consent.

Competing Interests

The authors declare that they have no competing interests.

References

1. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021 Oct;14(10):101174. doi: 10.1016/j.tranon.2021.101174. Epub 2021 Jul 6. PMID: 34243011; PMCID: PMC8273208.
2. Zhang Y, Devocelle A, Desterke C, de Souza LEB, Hadadi É, Acloque H, Foudi A, Xiang Y, Ballesta A, Chang Y, Giron-Michel J. BMAL1 Knockdown Leans Epithelial-Mesenchymal Balance toward Epithelial Properties and Decreases the Chemoresistance of Colon Carcinoma Cells. Int J Mol Sci. 2021 May 16;22(10):5247. doi: 10.3390/ijms22105247. PMID: 34065633; PMCID: PMC8157026.
3. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. Int J Mol Sci. 2017 Jan 19;18(1):197. doi: 10.3390/ijms18010197. PMID: 28106826; PMCID: PMC5297828.
4. Abdulla M. Management of Metastatic unresectable disease of colorectal cancer origin (Part 1: cytotoxic therapy). Res in Oncol. 2013;1(3-4):59-63.
5. Vukobrat-Bijedic Z, Husic-Selimovic A, Sofic A, Bijedic N, Bjelogrlic I, Gogov B, Mehmedovic A. Cancer Antigens (CEA and CA 19-9) as Markers of Advanced Stage of Colorectal Carcinoma. Med Arch. 2013 Dec;67(6):397-401. doi: 10.5455/medarh.2013.67.397-401. Epub 2013 Dec 28. PMID: 25568506; PMCID: PMC4272469.
6. Mostafa E F, Metwally A, Hussein S A. Inflammatory Bowel Diseases Prevalence in Patients Underwent Colonoscopy in Zagazig University Hospitals. Afro-Egypt J Infect Endem Dis. 2018; 8(2): 81-87. Doi: 10.21608/aeji.2018.8740.
7. Porter RJ, Kalla R, Ho GT. Ulcerative colitis: Recent advances in the understanding of disease pathogenesis. F1000Res. 2020 Apr 24;9:F1000 Faculty Rev-294. doi: 10.12688/f1000research.20805.1. PMID: 32399194; PMCID: PMC7194476.
8. Rubin DT, Ananthakrishnan AN, Siegel CA, Sauer BG, Long MD. ACG Clinical Guideline: Ulcerative Colitis in Adults. Am J Gastroenterol. 2019 Mar;114(3):384-413. doi: 10.14309/ajg.0000000000000152. PMID: 30840605.
9. Choi CR, Bakir IA, Hart AL, Graham TA. Clonal evolution of colorectal cancer in IBD. Nat Rev Gastroenterol Hepatol. 2017 Apr;14(4):218-229. doi: 10.1038/nrgastro.2017.1. Epub 2017 Feb 8. PMID: 28174420.
10. Yang X, Lou Y, Wang M, Liu C, Liu Y, Huang W. miR675 promotes colorectal cancer cell growth dependent on tumor suppressor DMTF1. Mol Med Rep. 2019 Mar;19(3):1481-1490. doi: 10.3892/mmr.2018.9780. Epub 2018 Dec 20. PMID: 30592263; PMCID: PMC6390018.
11. Yan J, Zhang Y, She Q, Li X, Peng L, Wang X, Liu S, Shen X, Zhang W, Dong Y, Lu J, Zhang G. Long Noncoding RNA H19/miR-675 Axis Promotes Gastric Cancer via FADD/Caspase 8/Caspase 3 Signaling Pathway. Cell Physiol Biochem. 2017;42(6):2364-2376. doi: 10.1159/000480028. Epub 2017 Aug 18. PMID: 28848149.
12. Li H, Li J, Jia S, Wu M, An J, Zheng Q, Zhang W, Lu D. miR675 upregulates long noncoding RNA H19 through activating EGR1 in human liver cancer. Oncotarget. 2015 Oct 13;6(31):31958-84. doi: 10.18632/oncotarget.5579. PMID: 26376677; PMCID: PMC4741653.
13. Wang J, Zhao YC, Lu YD, Ma CP. Integrated bioinformatics analyses identify dysregulated miRNAs in lung cancer. Eur Rev Med Pharmacol Sci. 2014 Aug;18(16):2270-4. PMID: 25219825.
14. Zhang Y, Huang W, Yuan Y, Li J, Wu J, Yu J, He Y, Wei Z, Zhang C. Long non-coding RNA H19 promotes colorectal cancer metastasis via binding to hnRNPA2B1. J Exp Clin Cancer Res. 2020 Jul 22;39(1):141. doi: 10.1186/s13046-020-01619-6. Erratum in: J Exp Clin Cancer Res. 2021 Mar 23;40(1):111. doi: 10.1186/s13046-021-01911-z. PMID:

32698890; PMCID: PMC7412843.
15. Ti Tili E, Michaille JJ, Piurowski V, Rigot B, Croce CM. MicroRNAs in intestinal barrier function, inflammatory bowel disease and related cancers-their effects and therapeutic potentials. Curr Opin Pharmacol. 2017 Dec;37:142-150. doi: 10.1016/j.coph.2017.10.010. Epub 2017 Nov 15. PMID: 29154194; PMCID: PMC5938753.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402-8. doi: 10.1006/meth.2001.1262. PMID: 11846609.
17. Kothari C R. Research methodology methods and techniques, 2nd ed New Age International Publishers. New Delhi, India; 2004.
18. Ahadi A. The significance of microRNA deregulation in colorectal cancer development and the clinical uses as a diagnostic and prognostic biomarker and therapeutic agent. Noncoding RNA Res. 2020 Aug 23;5(3):125-134. doi: 10.1016/j.ncrna.2020.08.003. PMID: 32954092; PMCID: PMC7476809.
19. Li J, Huang L, Zhao H, Yan Y, Lu J. The Role of Interleukins in Colorectal Cancer. Int J Biol Sci. 2020 Jun 14;16(13):2323-2339. doi: 10.7150/ijbs.46651. PMID: 32760201; PMCID: PMC7378639.
20. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, Sung JJY, Kaplan GG. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2017 Dec 23;390(10114):2769-2778. doi: 10.1016/S0140-6736(17)32448-0. Epub 2017 Oct 16. Erratum in: Lancet. 2020 Oct 3;396(10256):e56. doi: 10.1016/S0140-6736(20)32028-6. PMID: 29050646.
21. Zhang L, Zhou Y, Huang T, Cheng AS, Yu J, Kang W, To KF. The Interplay of LncRNA-H19 and Its Binding Partners in Physiological Process and Gastric Carcinogenesis. Int J Mol Sci. 2017 Feb 20;18(2):450. doi: 10.3390/ijms18020450. PMID: 28230721; PMCID: PMC5343984.
22. Sørensen CG, Karlsson WK, Pommergaard HC, Burcharth J, Rosenberg J. The diagnostic accuracy of carcinoembryonic antigen to detect colorectal cancer recurrence - A systematic review. Int J Surg. 2016 Jan;25:134-44. doi: 10.1016/j.ijsu.2015.11.065. Epub 2015 Dec 15. PMID: 26700203.
23. Chew MH, Koh PK, Ng KH, Eu KW. Improved survival in an Asian cohort of young colorectal cancer patients: an analysis of 523 patients from a single institution. Int J Colorectal Dis. 2009 Sep;24(9):1075-83. doi: 10.1007/s00384-009-0701-7. Epub 2009 Apr 23. PMID: 19387661.
24. González-Senac NM, Mayordomo-Cava J, Macías-Valle A, Aldama-Marín P, Majuelos González S, Cruz Arnés ML, Jiménez-Gómez LM, Vidán-Astiz MT, Serra-Rexach JA. Colorectal Cancer in Elderly Patients with Surgical Indication: State of the Art, Current Management, Role of Frailty and Benefits of a Geriatric Liaison. Int J Environ Res Public Health. 2021 Jun 4;18(11):6072. doi: 10.3390/ijerph18116072. PMID: 34199923; PMCID: PMC8200127.
25. El- Haddad S, Abdel Hady I, Sakr A, El-nahas T, Rashad A. Epidemiological Evaluation Focusing on Prognostic Impact of Young Age in Egyptian Patients with Colorectal Cancer, NEMROCK Experience Res Oncol. 2014;10(1-2): 6-11. doi: 10.21608/resoncol.2014.888
26. Kathpalia P, Sontag S. Age at IBD Diagnosis Does Not Impact Long-term Outcomes: 1785. Am J Gastroenterol. 2013; 108: S538-S539.
27. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017 Apr 29;389(10080):1756-1770. doi: 10.1016/S0140-6736(16)32126-2. Epub 2016 Dec 1. PMID: 27914657; PMCID: PMC6487890.
28. Kim SE, Paik HY, Yoon H, Lee JE, Kim N, Sung MK. Sex- and gender-specific disparities in colorectal cancer risk. World J Gastroenterol. 2015 May 7;21(17):5167-75. doi: 10.3748/wjg.v21.i17.5167. PMID: 25954090; PMCID: PMC4419057.
29. Matin F, Batra J. Profiling MicroRNA Markers in Plasma: Looking into Better Approaches and Recommendations. Methods Mol Biol. 2019;2054:93-103. doi: 10.1007/978-1-4939-9769-5_5. PMID: 31482449.
30. He D, Wang J, Zhang C, Shan B, Deng X, Li B, Zhou Y, Chen W, Hong J, Gao Y, Chen Z, Duan C. Down-regulation of miR-675-5p contributes to tumor progression and development by targeting pro-tumorigenic GPR55 in non-small cell lung cancer. Mol Cancer. 2015 Apr 1;14:73. doi: 10.1186/s12943-015-0342-0. PMID: 25889562; PMCID: PMC4392735.
31. Ren J, Fu J, Ma T, Yan B, Gao R, An Z, Wang D. LncRNA H19-elevated LIN28B promotes lung cancer progression through sequestering miR-196b. Cell Cycle. 2018;17(11):1372-1380. doi: 10.1080/15384101.2018.1482137. Epub 2018 Jul 23. PMID: 29950144; PMCID: PMC6110585.
32. Chen SW, Zhu J, Ma J, Zhang JL, Zuo S, Chen GW, Wang X, Pan YS, Liu YC, Wang PY. Overexpression of long non-coding RNA H19 is associated with unfavorable prognosis in patients with colorectal cancer and increased proliferation and migration in colon cancer cells. Oncol Lett. 2017 Aug;14(2):2446-2452. doi: 10.3892/ol.2017.6390. Epub 2017 Jun 15. PMID: 28781681; PMCID: PMC5530222.
33. Han D, Gao X, Wang M, Qiao Y, Xu Y, Yang J, Dong N, He J, Sun Q, Lv G, Xu C, Tao J, Ma N. Long noncoding RNA H19 indicates a poor prognosis of colorectal cancer and promotes tumor growth by recruiting and binding to eIF4A3. Oncotarget. 2016 Apr 19;7(16):22159-73. doi: 10.18632/oncotarget.8063. PMID: 26989025; PMCID: PMC5008352.
34. Ismail DM, Shaker OG, Kandeil MA, Hussein RM. Gene Expression of the Circulating Long Noncoding RNA *H19* and *HOTAIR* in Egyptian Colorectal Cancer Patients. Genet Test Mol Biomarkers. 2019 Sep;23(9):671-680. doi: 10.1089/gtmb.2019.0066. Epub 2019 Aug 21. PMID: 31433231.
35. Matouk IJ, Halle D, Raveh E, Gilon M, Sorin V, Hochberg A. The role of the oncofetal H19 lncRNA in tumor metastasis: orchestrating the EMT-MET decision. Oncotarget. 2016 Jan 26;7(4):3748-65. doi: 10.18632/oncotarget.6387. PMID: 26623562; PMCID: PMC4826167.
36. Ma L, Tian X, Guo H, Zhang Z, Du C, Wang F, Xie X, Gao H, Zhuang Y, Kornmann M, Gao H, Yang Y. Long noncoding RNA H19 derived miR-675 regulates cell proliferation by down-regulating E2F-1 in human pancreatic ductal adenocarcinoma. J Cancer. 2018 Jan 1;9(2):389-399. doi: 10.7150/jca.21347. PMID: 29344285; PMCID: PMC5771346.
37. Zou T., Jaladanki S.K., Liu L., Xiao L., Chung H.K., Wang J.Y., Xu Y., Gorospe M., Wang J.Y. H19 long noncoding RNA

regulates intestinal epithelial barrier function via microRNA 675 by interacting with RNAbinding protein HuR. Mol Cell Biol. 2016;36:1332–1341. doi: 10.1128/MCB.01030-15.
38. Lin L, Zhou G, Chen P, Wang Y, Han J, Chen M, He Y, Zhang S. Which long noncoding RNAs and circular RNAs contribute to inflammatory bowel disease? Cell Death Dis. 2020 Jun 15;11(6):456. doi: 10.1038/s41419-020-2657-z. PMID: 32541691; PMCID: PMC7295799.
39. Schneider C, Bodmer M, Jick SS, Meier CR. Colorectal cancer and markers of anemia. Eur J Cancer Prev. 2018 Nov;27(6):530-538. doi: 10.1097/CEJ.0000000000000397. PMID: 28692587.
40. Zhu X, Cao Y, Lu P, Kang Y, Lin Z, Hao T, Song Y. Evaluation of platelet indices as diagnostic biomarkers for colorectal cancer. Sci Rep. 2018 Aug 7;8(1):11814. doi: 10.1038/s41598-018-29293-x. PMID: 30087357; PMCID: PMC6081379.
41. Chen SW, Zhu J, Ma J, Zhang JL, Zuo S, Chen GW, Wang X, Pan YS, Liu YC, Wang PY. Overexpression of long non-coding RNA H19 is associated with unfavorable prognosis in patients with colorectal cancer and increased proliferation and migration in colon cancer cells. Oncol Lett. 2017 Aug;14(2):2446-2452. doi: 10.3892/ol.2017.6390. Epub 2017 Jun 15. PMID: 28781681; PMCID: PMC5530222.
42. Rogler G, Vavricka S. Anemia in inflammatory bowel disease: an under-estimated problem? Front Med (Lausanne). 2015 Jan 19;1:58. doi: 10.3389/fmed.2014.00058. PMID: 25646159; PMCID: PMC4298217.
43. Ananthakrishnan AN, Cheng SC, Cai T, Cagan A, Gainer VS, Szolovits P, Shaw SY, Churchill S, Karlson EW, Murphy SN, Kohane I, Liao KP. Serum inflammatory markers and risk of colorectal cancer in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol. 2014 Aug;12(8):1342-8. e1. doi: 10.1016/j.cgh.2013.12.030. Epub 2014 Jan 7. PMID: 24407106; PMCID: PMC4085150.
44. Wang A, Jiang Y. Correlation analysis of serum levels of H19 and CRP levels and ulcerative colitis. J Med Biochem. 2023 Aug 25;42(3):420-426. doi: 10.5937/jomb0-41359. PMID: 37814617; PMCID: PMC10560500.
45. Shaker OG, Safa A, Khairy A, Abozeid NF. Serum long noncoding RNA H19/micro RNA-675-5p axis as a probable diagnostic biomarker in inflammatory bowel disease. Mol Biol Rep. 2023 Nov;50(11):9029-9036. doi: 10.1007/s11033-023-08777-8. Epub 2023 Sep 16. PMID: 37716920; PMCID: PMC10635930.

***Corresponding author**: Dalia A. Gaber, Associate professor of Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Helwan University, Cairo, Egypt. College of Medicine, Gulf Medical University, Ajman, United Arab Emirates. E-mail: dalia.ali@med.helwan.edu.eg*