RESEARCH ARTICLE

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Combined Circulating miR-17-5p and Heat Shock Proteins as Diagnostic Biomarkers for Colorectal Cancer Progression

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Abstract

Objectives: Colorectal cancer (CRC) remains a significant global health challenge, and early diagnosis is essential for improving patient survival and treatment outcomes. Currently used serum biomarkers, including carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), suffer from limited sensitivity and specificity, particularly in early-stage disease, which restricts their clinical utility. Therefore, identifying more reliable biomarkers for CRC detection and monitoring is urgently needed. This study investigated the diagnostic value of microRNA-17-5p (miR-17-5p) and selected heat shock proteins (HSPs) as potential non-invasive biomarkers for CRC. **Methods:** The study included patients with confirmed CRC, individuals with benign colorectal diseases, and healthy control subjects. Plasma levels of miR-17-5p and relative gene expression of HSPs were quantified in obtained samples using quantitative real-time PCR, while circulating HSP concentrations were measured using enzyme-linked immunosorbent assay (ELISA). **Results:** The results demonstrated a marked upregulation of miR-17-5p in CRC patients, showing a six-fold increase compared with healthy controls. Patients with benign colorectal disorders exhibited a moderate two-fold increase. Similarly, *HSP40* and HSP90 expression levels were significantly elevated in CRC patients, whereas their levels were reduced in benign colorectal cases relative to healthy individuals. In contrast, *HSP70* expression was significantly decreased in both CRC and benign colorectal patients compared with controls. Overall, miR-17-5p and HSPs showed superior diagnostic accuracy compared with conventional biomarkers. Notably, combined biomarker analysis significantly enhanced sensitivity and specificity in distinguishing CRC from benign colorectal conditions. Among the evaluated markers, miR-17-5p in combination with *HSP40* and HSP90 demonstrated the strongest diagnostic performance. **Conclusion:** These findings suggest that this biomarker panel holds promise for CRC diagnosis, prognosis, and potential therapeutic targeting, offering improved strategies for clinical management.

Keywords: Colorectal cancer, miR-17-5p, Heat shock proteins, Diagnostic biomarkers

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide and the second leading cause of cancer-related mortality, predominantly affecting individuals over the age of 50 [1, 2]. It is characterized by abnormal epithelial cell proliferation within the colon and gastrointestinal tract. Metastasis, particularly to the liver, lungs, bones, brain, and spinal cord, represents the primary cause of death among CRC patients [3]. The risk of CRC is influenced by multiple factors, including genetic predisposition, family history, and lifestyle choices Major risk factors include hereditary syndromes, gene mutations, an imbalanced gut microbiome, obesity, smoking, alcohol consumption, a high-fat and low-fiber diet, inflammatory bowel disease (IBD), adenomatous polyps, and diabetes [4, 5]. CRC commonly arises from benign polyps in the large intestine that may transform into malignant lesions under conditions of chronic inflammation. Although treatment strategies have advanced, survival outcomes remain highly dependent on early detection and timely intervention [6]. Because early-stage CRC is often asymptomatic or presents with vague, non-specific symptoms, routine screening and early diagnosis are crucial for improving patient prognosis [7].

Current diagnostic approaches, including colonoscopy and histopathological analysis, remain the gold standard for CRC detection [8]. However, these procedures

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are invasive, costly, and unsuitable for large-scale or routine population screening [9]. Consequently, non-invasive diagnostic alternatives, particularly blood-based biomarkers, have gained significant attention [10]. Conventional serum biomarkers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are widely used for CRC diagnosis and monitoring [11]. Nevertheless, their clinical utility is limited by low sensitivity and specificity, especially in early-stage disease [12]. Moreover, elevated levels of these markers are not exclusive to CRC and may also occur in benign conditions such as inflammatory bowel disease, pancreatitis, and other malignancies [13]. This highlights the urgent need for novel and highly specific biomarkers to enhance the accuracy of CRC detection, particularly at early stages.

Recently, microRNAs (miRNAs) and heat shock proteins (HSPs) have emerged as promising non-invasive biomarkers for cancer detection, including CRC [14, 15]. miRNAs are small, non-coding RNAs that regulate gene expression at the post-transcriptional level, influencing key biological processes such as cell proliferation, apoptosis, and metastasis. Dysregulated miRNA expression has been strongly implicated in cancer development, with several miRNAs identified as potential diagnostic and prognostic biomarkers [16–18]. Among these, miR-17-5p is widely recognized as an oncogenic miRNA that promotes tumor growth, angiogenesis, and resistance to apoptosis [19, 20]. Upregulation of miR-17-5p has been observed in CRC, and its elevated expression is associated with poor prognosis, underscoring its potential diagnostic and prognostic value [21, 22].

Similarly, HSPs are molecular chaperones that are upregulated in response to cellular stress [23, 24]. They play a crucial role in protein folding, stabilization, and protection against stressors [25, 26]. Overexpression of HSPs has been reported in several cancers, including CRC, where they contribute to tumor progression, immune evasion, and resistance to apoptosis [27, 28]. These properties highlight their potential utility as biomarkers for CRC diagnosis and monitoring.

Although miR-17-5p and HSPs have each shown diagnostic promise independently, their potential combined role and molecular interplay in CRC remain largely unexplored. Given their distinct yet complementary contributions to cancer pathophysiology, integrating these markers into a unified diagnostic framework may enhance the sensitivity and specificity of CRC detection. Therefore, the present study aimed to investigate the diagnostic potential of miR-17-5p and HSPs as non-invasive biomarkers for CRC.

Materials and Methods

Study design

This study was conducted between 15 January 2023 and 15 January 2025 at Shifaa Al Orman Oncology Hospital, Luxor, Egypt. Prior to enrollment, all participants provided written informed consent, ensuring voluntary participation and compliance with ethical standards. A total of 135 individuals were recruited and divided into three groups: 50 patients with CRC, 45 patients with benign colorectal conditions, and 40 healthy controls. Recruitment was carried out through the surgical oncology outpatient clinics at Shifaa Al Orman Oncology Hospital. The study protocol followed the ethical principles of the Declaration of Helsinki and was approved by the Institutional Review Board (SOH-IRB) of Shifaa Al Orman Oncology Hospital (Approval ID: 1-2024).

Inclusion and exclusion criteria

Participants were eligible for inclusion if they had histopathologically confirmed CRC at various tumor stages, benign colorectal conditions such as colorectal polyps or inflammatory bowel disease, or were healthy controls without a history of malignancy, systemic inflammatory disorders, or gastrointestinal diseases. Exclusion criteria included individuals with a prior history of other cancers, to avoid potential biomarker cross-reactivity; those who had undergone chemotherapy or radiotherapy within six months before enrollment, to eliminate treatment-related effects on biomarker expression; and individuals with autoimmune or chronic inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, or Crohn's disease, which could influence biomarker levels. Patients with severe metabolic disorders or recent infections were also excluded to minimize confounding variations related to inflammation or stress responses. These criteria were established to ensure a well-defined study population and to enhance the reliability and validity of biomarker evaluation.

Sample size and condition

The required sample size was determined using GPower software (Version 3.1, Heinrich-Heine University, Düsseldorf, Germany). Based on an anticipated effect size of 0.6, derived from previous studies on circulating miRNAs and HSPs in colorectal cancer (CRC), the minimum number of participants per group was calculated to achieve 80% statistical power at a 95% confidence level. The GPower analysis confirmed that the selected sample size was sufficient to ensure adequate statistical power and enhance the reliability and accuracy of the study outcomes [29]. Venous blood samples were collected from all participants in sterile EDTA-coated tubes to prevent coagulation. Samples were immediately placed on ice and processed within 2 hours to preserve biomarker stability. Plasma separation was performed by centrifugation at 3,000 rpm for 10 minutes at 4°C. The supernatant was then carefully transferred into DNase- and RNase-free microcentrifuge tubes and subjected to a second centrifugation at 12,000 rpm for 10 minutes at 4°C to eliminate residual debris and hemolysis-related contaminants. Plasma samples were subsequently aliquoted and stored at −80°C until RNA and protein extraction to maintain sample integrity [30].

Small RNA purification and complementary DNA generation

Total RNA, including small RNAs, was extracted from plasma samples using the TRIzol LS Reagent (Invitrogen, Thermo Fisher Scientific, USA, Catalog No. 10296028)

following the manufacturer's protocol. In brief, in 2 mL of DNase-RNase free eppendorf tubes, an equal volume TRIzol was added to each sample followed by gentle vortexing. 500 µL of chloroform was added to each tube with a gentle vortexing and centrifugation under cooling for 20 minutes at 12,000 rpm. The supernatant was carefully transferred to a clean eppendorf tubes, and 500 isopropanol was added to each taubes, and then total RNA was participated by centrifuging the samples under cooling at 12,000 rpm for 10 minutes [31]. The RNA pellet was washed with 75% ethanol, air-dried, and resuspended in 50 µL DNase-RNase-free water. Total RNA concentration and purity were assessed using a Qubit 4 Fluorometer (Thermo Fisher Scientific, USA) with the RNA High Sensitivity Assay Kit (Catalog No. Q32852). To generate the complementary DNA (cDNA), miRNA reverse transcription was carried out using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, Catalog No. A28007). The reaction included poly(A) tailing, adapter ligation, and reverse transcription, ensuring high efficiency and specificity.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed on a Bio-Rad CFX96 Touch Real-Time PCR System using TaqMan™ Advanced miRNA Assays (Applied Biosystems, Catalog No. A25576) with miR-17-5p-specific primers listed in Table 1 [32]. Each reaction was carried out in a 20 µL volume containing 10 µL TaqMan™ Fast Advanced Master Mix (Applied Biosystems, Catalog No. 4444557), 2 µL diluted cDNA, 1 µL miR-17-5p-specific TaqMan probe, and 7 µL nuclease-free water. The thermal cycling conditions were Initial denaturation at 95°C for 2 minutes. 40 cycles of denaturation at 95°C for 5 seconds. Annealing/extension at 60°C for 30 seconds. The relative expression levels of miR-17-5p were normalized to U6 small nuclear RNA (snRNA) as an internal reference. The comparative $2^{-\Delta\Delta Ct}$ method was used to quantify the fold change in expression between CRC, benign, and control groups [33]. All reactions were performed in triplicate to ensure reproducibility.

Gene expression profiling

The relative expression levels of HSP genes, including *HSP40*, *HSP70*, and HSP90, were analyzed using quantitative qRT-PCR in the collected samples. The synthesis and amplification of cDNA were performed in a one-step reaction using purified total RNA as a template. Total RNA was extracted immediately after sample collection using TRIzol reagent and subsequently purified with the RNeasy Mini Kit (Qiagen, USA). The relative expression of *HSP40*, *HSP70*, and HSP90 was quantified using the QuantiTect SYBR Green PCR Kit (Qiagen, USA). Gene-specific primers listed in Table 1 were used for amplification, and GAPDH served as the internal normalization control. Each reaction contained the following components: 10 µL SYBR Green master mix, 0.2 µL RNase inhibitor (20 U/µL), 0.25 µL reverse transcriptase (50 U/µL), 1 µL purified total RNA (100 ng/µL), and 0.5 µL of each primer, with RNase-free water added to a final volume of 20 µL.

Table 1. Oligonucleotide Sequences Used for the Quantification of miR-17-5p and HSP mRNA in Derived Samples

Description	Primer sequences 5'-3'
MiR-17-5P-sense	TGCGCCAAAGTGCTTACAGTGCA
MiR-17-5P antisense	CCAGTGCAGGGTCCGAGGTATT
U6-sense	GCTTCGGCAGCACATATACTAAAAT
U6-antisense	CGCTTCACGAATTTGCGTGTCAT
HSP40-sense	TCGCAAGCATACGAAGTTCTCAGC
HSP40-antisense	CCGAAGAACATGTCGAAGATGTCC
HSP70-sense	ATGTCGGTGGTGGGCATAGA
HSP70-antisense	CACAGCGACGTAGCAGCTCT
HSP90-sense	CGAGTACCTGAACTTCCTCAATGG
HSP90-antisense	CCTGTTCACGAGATTCACCAGTGA
GAPDH-sense	TGGCATTGTGGAAGGGCTCA
GAPDH-antisense	TGGATGCAGGGATGATGTTCT

The one-step qRT-PCR reaction was performed according to the manufacturer's protocol under the following thermal cycling conditions: 50 °C for 45 min, 95 °C for 4 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 15 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min [34, 35].

HSP quantification via ELISA

HSP plasma levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits. HSP detection was carried out using the Enzo Life Sciences ELISA Kit (Farmingdale, NY, USA, Catalog No. ADI-EKS-715). Plasma samples were diluted in assay buffer and added to microtiter plates pre-coated with monoclonal antibodies specific for HSPs. After incubation and washing, horseradish peroxidase (HRP)-conjugated secondary antibodies were applied. The colorimetric reaction was developed and measured at 450 nm using a Bio-Tek

Table 2. Demographic Comparison of CRC, benign, and Control Groups

Demographic Characteristic	CRC Group (n=50)	Benign Group (n=45)	Control Group (n=40)	Statistical Test	p-value
Age (Mean ± SD)	62.3 ± 8.2	55.7 ± 7.3	58.5 ± 6.1	ANOVA with Tukey's	0.042*
Sex: (Male)	28 (56%)	20 (44%)	22 (55%)	Chi-Square	0.151
(Female)	22 (44%)	25 (56%)	18 (45%)		
Smoking History	30 (60%)	15 (33%)	12 (30%)	Chi-Square	0.002*
Comorbidities (Hypertension)	25 (50%)	18 (40%)	15 (37.5%)	Chi-Square	0.221
Comorbidities (Diabetes)	15 (30%)	10 (22%)	8 (20%)	Chi-Square	0.358

CRC, colorectal cancer; n, number of samples

Table 3. Descriptive Statistical Analysis of CA19-9, CEA, and miR-17-5p Across Control, Benign, and CRC Groups

Marker	Group	Mean ± SD	Min	Max	p-value
CA19-9 (U/mL)	CRC	45.2 ± 12.3	15	225	0.032*
	Benign	40.0 ± 15.0	10	150	
	Control	20.0 ± 5.0	5	50	
CEA (ng/mL)	CRC	5.6 ± 3.1	1	19	0.217 (NS)
	Benign	4.0 ± 2.5	1	10	
	Control	2.5 ± 1.2	0.5	5	
miR-17-5p (fold)	CRC	1.4 ± 0.7	0.5	3	0.005*
	Benign	1.1 ± 0.5	0.3	2.5	
	Control	0.8 ± 0.4	0.2	1.5	
HSP (pg/mL)	CRC	250.4±13.2			0.021*
	Benign	175.2±10.2			
	Control	38.25±2.3			

CA19-9, Carbohydrate antigen 19-9; CEA, Carcinoembryonic antigen; CRC, Colorectal cancer; HSP, Heat shock protein.

Synergy HTX microplate reader (Bio-Tek Instruments, Winooski, VT, USA) [36].

Data analysis

Data normality was evaluated using the Kolmogorov–Smirnov and Shapiro–Wilk tests. For normally distributed variables, one-way analysis of variance (ANOVA) followed by Bonferroni post hoc comparisons was applied. For non-normally distributed data, the Kruskal–Wallis test with Dunn's post hoc analysis was used. The diagnostic performance of miR-17-5p and heat shock proteins (HSPs) was assessed through receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to determine their diagnostic accuracy for colorectal cancer (CRC). For gene expression quantification, the cycle threshold (Ct) values were analyzed using the comparative delta-delta-Ct (ΔΔCt) method as previously described. The calculations were performed as follows: ΔCt = Ct value of the target gene − Ct value of GAPDH. ΔΔCt = ΔCt (experimental group) − ΔCt (control group). Relative gene expression = $2^{(-\Delta\Delta Ct)}$. Similarly, for miR-17-5p expression quantification, the ΔΔCt method was applied: ΔCt = Ct value of miR-17-5p − Ct value of U6 snRNA. ΔΔCt = ΔCt (experimental group) − ΔCt (control group). Relative expression (upregulation) of miR-17-5p = $2^{(-\Delta\Delta Ct)}$. Statistical analysis was performed using the student's t-test to compare each cancer group with the healthy control group. A p-value ≤ 0.05 was considered statistically significant [37, 38].

Results

Demographic and clinical characteristics of CRC patients, benign cases, and healthy controls

The demographic and clinical characteristics of the study population are summarized in Table 2. The CRC group had a significantly higher mean age (62.3 ± 8.2 years) compared with the benign (55.7 ± 7.3 years) and control (58.5 ± 6.1 years) groups (p = 0.042). The sex distribution did not differ significantly among groups, with males comprising 56% of CRC patients, 44% of the benign group, and 55% of healthy controls (p = 0.151). A significantly greater proportion of CRC patients were smokers (60%) compared to the benign (33%) and control (30%) groups (p = 0.002). In contrast, comorbidities such as hypertension and diabetes showed no significant differences among the three groups (p > 0.05). The levels of tumor and molecular markers, CA19-9, CEA, miR-17-5p, and HSP, are summarized in Table 3. CA19-9 levels were markedly higher in CRC patients (45.2 ± 12.3 U/mL) compared to controls (20.0 ± 5.0 U/mL, p = 0.032), with the benign group showing intermediate levels (40.0 ± 15.0 U/mL). miR-17-5p expression was significantly upregulated in CRC patients (1.4 ± 0.7-fold) compared to the benign (1.1 ± 0.5-fold) and control (0.8 ± 0.4-fold) groups (p = 0.005). Similarly, HSP levels were substantially higher in CRC patients (250.4 ± 13.2 pg/mL) compared with the benign (175.2 ± 10.2 pg/mL) and control (38.25 ± 2.3 pg/mL) groups (p = 0.021). In contrast, CEA levels did not differ significantly among the three groups (p = 0.217). Tumor staging data (Table 4) revealed that CRC patients were distributed across Stages

Table 4. Comparison of Tumor Stage Distribution between CRC, benign, and Control Groups (Chi-Square Test)

Tumor Stage	CRC Group (n=50)	Benign Group (n=45)	Control Group (n=40)	Chi-Square	p-value
Stage 0 (No Tumor)	0	35	40	45.23	<0.001*
Stage I	12	5	0		
Stage II	18	3	0		
Stage III	15	2	0		
Stage IV	5	0	0		

CRC, colorectal cancer; n, number of samples

I–IV, with 24% in Stage I, 36% in Stage II, 30% in Stage III, and 10% in Stage IV. Conversely, 77.8% of the benign group and all control subjects (100%) were classified as benign lesions ($p < 0.001$).

Diagnostic and predictive significance of miR-17-5p, HSP, and their composite score in CRC

Early and accurate detection of CRC remains a major challenge, emphasizing the need for reliable molecular and clinical biomarkers. Here, the diagnostic potential of miR-17-5p and HSP mRNA was evaluated individually and in combination. As shown in Figure 1, the ROC curve analysis demonstrated that miR-17-5p exhibited strong diagnostic performance with an AUC of 0.844, indicating its high ability to discriminate between CRC patients and controls. In contrast, HSP mRNA showed a lower AUC of 0.723, reflecting moderate diagnostic capacity. Notably, the combined score, integrating miR-17-5p and HSP expression data, achieved the highest AUC of 0.900, confirming superior diagnostic accuracy compared with either marker alone Table 5. These findings suggest that miR-17-5p serves as a more reliable biomarker than HSP mRNA, and that their combination significantly enhances diagnostic precision and predictive value. Logistic regression analysis (Supplementary Table 1) further supported these results, identifying miR-17-5p (OR = 2.75, $p < 0.001$), HSP (OR = 1.98, $p = 0.002$), and their composite score (OR = 3.10, $p < 0.001$) as independent predictors of CRC. Among clinical variables, older age (OR = 1.05, $p = 0.005$) and smoking history (OR = 2.50, $p = 0.029$) were also significantly associated with increased CRC risk. Pearson's correlation analysis (Supplementary Table 2) revealed strong positive correlations between the composite score and both miR-17-5p ($r = 0.85$, $p < 0.001$) and HSP ($r = 0.72$, $p < 0.001$), confirming the close relationship between these biomarkers. Additionally, age ($r = 0.41$, $p < 0.01$) and smoking history ($r = 0.45$, $p < 0.001$) showed moderate correlations with the composite score, indicating their role as contributing factors in CRC development. The strong correlations underscore the clinical and statistical robustness of integrating miR-17-5p and HSP into a single diagnostic model. A composite model incorporating miR-17-5p, HSP, age, and smoking history achieved an AUC of 0.94 (95% CI: 0.89–0.98), with 91% sensitivity and 92% specificity at a cut-off score of 5.0 ($p < 0.001$, Supplementary Table 3).

miR-17-5p expression is elevated alongside increased HSP40 and HSP90 levels in CRC samples

The expression profile of miR-17-5p and HSPs was monitored in samples derived from CRC patients, benign tumor patients, and healthy controls using qRT-PCR. Interestingly, the relative expression level of miR-17-5p was significantly upregulated, showing approximately a 5-fold increase in CRC patient samples compared with healthy controls (Figure 2A; Supplementary Table 4). Similarly, samples from benign tumor patients also exhibited a significant increase in miR-17-5p expression, although to a lesser extent, reaching approximately a 2.5-fold increase relative to healthy controls. In parallel, the relative gene expression of *HSP40* was markedly elevated in CRC-derived samples, showing more than a 5-fold increase, while HSP90 expression was increased by over 4-fold compared with healthy controls (Figure 2B; Supplementary Table 5). In contrast, the relative expression of *HSP70* was significantly decreased in samples derived from both CRC and benign tumor patients compared with healthy controls (Figure 2B; Supplementary Table 5). Collectively, these findings indicate that miR-17-5p is markedly upregulated in colon tumors, including both benign and malignant forms, with a twofold higher expression in CRC compared to benign tumors.

Discussion

Our study underscores the diagnostic significance of miR-17-5p and HSPs as promising biomarkers for CRC, enabling clearer differentiation between malignant,

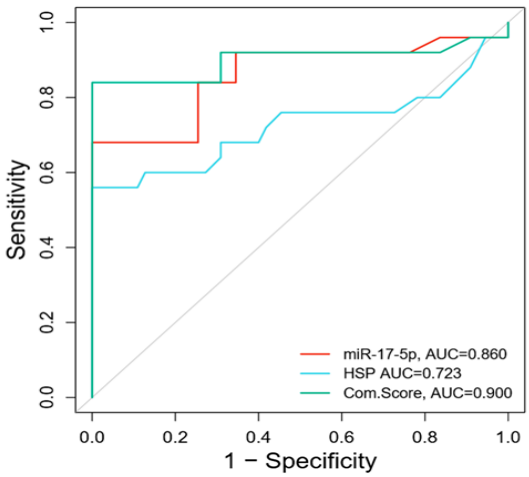

Figure 1. Receiver Operating Characteristic (ROC) curves for miR-17-5p, HSP mRNA, and their combined diagnostic score. ROC curves illustrating the diagnostic performance of miR-17-5p (red), HSP mRNA (light blue), and their combined score (green) in distinguishing the study groups. The area under the curve (AUC) values were 0.860 for miR-17-5p, 0.723 for HSP mRNA, and 0.900 for the combined score (Com. Score), respectively. The diagonal grey line represents the reference line for random classification (AUC = 0.5). Higher AUC values indicate better diagnostic accuracy.

Table 5. ROC Curve Analysis for miR-17-5p, HSP, and Composite Marker

Marker	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Cut-off Point	p-value (vs. Control)
miR-17-5p	0.844 (0.82-0.93)	82	85	2.4	< 0.001**
HSP	0.791 (0.68-0.82)	75	72	1.2	< 0.05*
miR-17-5p + HSP (Composite)	0.90 (0.88-0.96)	89	90	3	< 0.001**

AUC, Area Under the Curve; RCO, Receiver Operating Characteristic; HSP, Heat shock protein

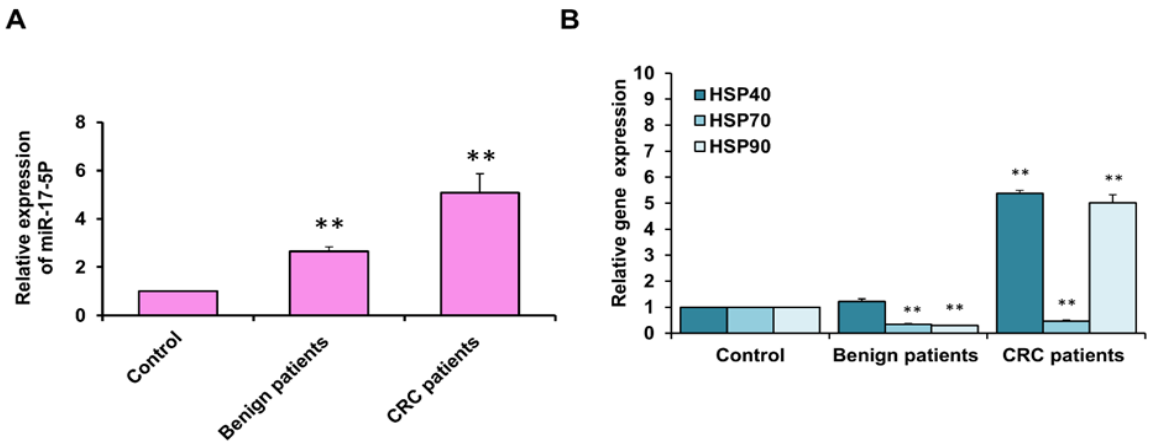

Figure 2. Relative expression of miR-17-5p and HSPs in patient-derived samples. (A) Quantitative analysis of miR-17-5p expression in samples derived from colorectal cancer (CRC) patients, benign tumor patients, and healthy controls, as determined by qRT-PCR and expressed as fold change. (B) Relative gene expression levels of HSP40, HSP70, and HSP90 in samples derived from CRC patients, benign tumor patients, and healthy controls, as determined by qRT-PCR and expressed as fold change. Error bars represent the standard deviation (SD) from three independent experiments. Statistical significance was assessed using a two-tailed Student's t-test; ** indicates $P \leq 0.01$.

benign, and healthy cases. Demographically, CRC patients were older, with most cases occurring in individuals over 60 years, and smoking history was significantly associated with CRC ($p = 0.002$), consistent with evidence linking tobacco exposure to carcinogenesis through chronic inflammation [39, 40]. In evaluating conventional tumor markers, CA19-9 levels were found to be significantly elevated in CRC patients compared with controls. This finding is consistent with previous studies reporting frequent CA19-9 elevation in CRC; however, its diagnostic specificity remains limited, as the marker can also be increased in other gastrointestinal conditions [41]. Moreover, our data question the reliability of CA19-9 for early-stage CRC detection, emphasizing the need for incorporating additional biomarkers to enhance diagnostic precision. Similarly, CEA levels did not differ significantly among CRC, benign, and control groups, reinforcing concerns regarding its low sensitivity in early-stage disease. These results suggest that while CEA may be valuable for disease monitoring, it is less effective as a primary diagnostic indicator [42, 43].

A key finding of this study was the significant upregulation of miR-17-5p in CRC patients relative to benign and control groups, supporting its role as a potential oncogenic biomarker. Previous research has shown that miR-17-5p contributes to CRC progression by modulating tumor suppressor pathways, including PTEN and TGF-β, thereby promoting cell proliferation and survival [44, 45]. Unlike earlier studies focusing solely on malignant cases [46–48] our inclusion of benign cases strengthens evidence for its specificity in distinguishing malignant from non-malignant conditions. Additionally, HSP levels were markedly elevated in CRC patients compared with both benign and control groups. HSPs are known to contribute to CRC pathogenesis by inhibiting apoptosis and promoting tumor survival through activation of AKT and NF-κB signaling pathways [49, 50]. Unlike prior studies that reported increased HSP expression primarily in advanced-stage CRC [51, 52], our results demonstrate their elevation in early-stage disease, highlighting their potential as early detection biomarkers. Our findings further demonstrate that, in contrast to *HSP40* and HSP90, *HSP70* exhibited a lower expression profile at the same time point of measurement. This observation suggests that *HSP70* may be negatively regulated by the upregulated levels of miR-17-5p. Previous studies have reported an association between serum *HSP70* levels and colorectal cancer mortality. This relationship may be partially explained by the regulatory effect of miR-17-5p on *HSP70* during early-stage disease, an effect that appears to diminish in advanced and terminal stages of CRC [53]. Indeed, *HSP70* plays a critical role in CRC progression by functioning as a molecular chaperone that stabilizes protein structure, facilitates the repair or degradation of damaged proteins, and maintains cellular protein homeostasis. Therefore, the potential targeting of *HSP70* by miR-17-5p during the early stages of CRC may contribute to tumor progression and evolution.

A notable strength of this work lies in the combined evaluation of miR-17-5p and HSPs as a biomarker panel. ROC curve analysis revealed that miR-17-5p alone achieved an AUC of 0.844 (sensitivity 82%, specificity 85%), whereas the combined panel improved diagnostic performance, yielding an AUC of 0.90 with 89% sensitivity and 90% specificity. This synergistic enhancement surpasses traditional markers such as CEA and CA19-9, underscoring the promise of a multi-marker approach for accurate and reliable CRC detection. In summary, the marked upregulation of miR-17-5p in CRC may contribute to tumor progression by suppressing tumor suppressor pathways, enhancing heat shock protein activity, and promoting cancer cell proliferation, migration, and resistance to therapy. These findings underscore the pivotal role of miR-17-5p as a key molecular regulator in CRC pathogenesis and highlight its potential as a valuable biomarker and therapeutic target for improved diagnosis and treatment strategies. Several important limitations of this study should be acknowledged. First, the

relatively limited number of participants and the lack of representation from broader and more diverse populations may restrict the generalizability of the findings. Second, the assessment of biomarkers at a single time point limits the ability to evaluate their dynamic changes over time, as well as their potential role in monitoring disease progression, recurrence, or response to treatment. In addition, the absence of direct comparison between HSPs, miR-17-5p, and established standard screening modalities, such as colonoscopy or imaging techniques, may reduce the immediate clinical applicability of the results. Finally, the potential influence of miR-17-5p on other biological processes, such as inflammation, other malignancies, systemic diseases, and medication effects, represents another limitation, as these factors could confound its specificity as a biomarker for colorectal cancer and should be further investigated.

In conclusion, this study investigates the diagnostic potential of miR-17-5p and HSPs as alternative biomarkers for CRC. Our findings identify miR-17-5p and HSPs as promising diagnostic biomarkers for CRC, exhibiting higher diagnostic accuracy compared with conventional markers. Notably, the combined evaluation of miR-17-5p with HSPs enhances the sensitivity and specificity of early CRC detection, providing a more robust approach to differentiate malignant from non-malignant cases. Further analysis revealed that *HSP40* and HSP90, in particular, may serve as complementary biomarkers alongside miR-17-5p, collectively offering a sensitive diagnostic panel for CRC progression. Collectively, these findings support the hypothesis that miR-17-5p functions as a key biomarker for both CRC diagnosis and disease progression and further suggest that therapeutic targeting of miR-17-5p may offer a novel strategy to improve clinical outcomes in CRC patients.

Author Contribution Statement

H.A., M.N., A.Z., N.R., and A.T. conducted the experiments. K.B. assisted in supervising and conceptualizing the experiments. H.K. developed the research plan, led the overall study, analyzed and interpreted the results. H.K. organized and wrote the manuscript..

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None.

Ethical Declaration

Ethics approval and consent of participants

Ethical approval for this study was granted by the Ethics Committee of Shefaa Al Orman Hospital, Egyptian Ministry of Health and Population (Ethical Code: RHDIRB2017061502), and by the Ethics Committee of the Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt (Ethical Code: SRE060725B10063). Informed consent was obtained from all participants after a full explanation of the study's objectives and procedures.

Consent for publication

All authors read the manuscript and approved publication.

Availability of data and materials

The data supporting these findings are included in the main manuscript.

Conflicting interests

All authors declare that there are no conflicts of interest.

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