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Dysregulation of *miR-205*, *BCL2*, and *YAP1* as molecular signatures of colorectal cancer in Iraqi patients: a case–control study

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Abstract

Colorectal Cancer (CRC) is one of the most common malignancies globally, and its increasing prevalence in Iraq is an emerging health problem. This work analyzed the expression profiles of *miR-205* (microRNA-205), *BCL2* (B-cell lymphoma 2), and *YAP1* gene in Iraqi CRC patients to evaluate the potential contribution towards diagnosis and prognosis. Eighty individual CRC and 40 healthy Control patients were recruited. Complete Blood Count (CBC) analysis was performed to assess White Blood Cell (WBC) concentrations, and Interleukin-18 (IL-18) and Interleukin-22 (IL-22) concentrations were determined by Enzyme-Linked Immunosorbent Assay (ELISA). Quantitative real-time Polymerase Chain Reaction (qRT-PCR) was utilized to quantify expression levels of *miR-205, BCL2,* and *YAP1*. Diagnostic efficacy was established by performing Receiver

Operating Characteristic (ROC) curve analysis, and biomarker associations were evaluated using Pearson correlation. Differences in WBC count levels, IL-18, and IL-22 levels were non-significant in patients *vs*. controls. In contrast, *miR-205* expression was significantly decreased in CRC patients (0.33-fold), whereas *BCL2* and *YAP1* were significantly up-regulated (1.92-fold and 1.44-fold, respectively). ROC analysis revealed that *BCL2* showed the greatest diagnostic activity, with an area under the curve (AUC) of 0.880, and was followed by *YAP1* (AUC: 0.736) and *miR-205* (AUC: 0.700). There were significant correlations between *miR-205*/*BCL2* and *BCL2*/*YAP1*, indicating coordinated roles in apoptosis blocking and tumor growth mechanisms. Collectively these results recommend the tumor suppressive role of *miR-205* and the oncogenic role of *BCL2* and *YAP1* which could explain CRC phenotypes and therapy resistance. Overall, the study d=also suggest the potential of these genes as cancer detection candidate in the Iraqi population even within its limitations.

Introduction

Colorectal Cancer (CRC) is one of the most prevalent tumors worldwide, which is the 3rd most common cancer in men and the 2nd most common cancer in women. It is also among the leading causes cancer-related mortality (responsible for about 9.4% of cancer deaths globally).[1] The trend in the incidence of CRC has been increasingly rising in Iraq, constituting a major public health issue requiring more detailed molecular studies on its molecular basis.[2] CRC development is a complex interplay of genetic, epigenetic, and environmental influences. Of these molecular regulators, a growing amount od attention is focused on microRNAs (miRNAs) that act to target mRNAs in post-transcriptional manners that may affect different biochemical processes such as cell proliferation, differentiation and apoptosis.[3] One of those miRNAs relevant to CRC, *miR-205*, is characterized as both an oncogene and tumor suppressor, depending on the cellular context.[4] Data from the Iraqi Cancer Registry reveal a significant upward trend for CRC. While it ranked third in 2020 with 2,210 cases (5.34% prevalence), it climbed to the second most common cancer by 2022, reaching 2,871 cases and a prevalence of 7.3%.[5,6]

BCL2 (B-cell lymphoma-2) is an essential anti-apoptotic protein and vital to cell survival and oncogenesis. It controls the intrinsic apoptotic cascade by blocking cytochrome c release from mitochondria, which is the prerequisite for caspase activation of cell death. *BCL2* overexpression is significantly reported in various cancers, including CRC, and promotes tumor resistance against

chemotherapeutic agents.[7] In CRC patients, increased levels of *BCL2* have been correlated with poor outcome and resistance to apoptosis-inducing drugs, so it has been proposed as a target for cancer therapy.[8]

Another prominent player in CRC is Yes-Associated Protein 1 (YAP1), a transcriptional co-activator and master effector of the Hippo signaling pathway. *YAP1* is a key regulator of organ size, stem cell renewal, and tumor genesis.[9] Hippo pathway dysregulation causes elevated nuclear accumulation of *YAP1*, resulting in cellular proliferation, invasion, and metastasis in diverse cancers, including CRC.[10] *YAP1* can be aberrantly regulated to activate oncogenic signaling pathways, including Wnt/β-catenin, which is the major driving force of colorectal tumor genesis.[11] Given the critical involvement of *miR-205, BCL2,* and *YAP1* in the pathogenesis of CRC, this study aims to quantify their expression profiles within the Iraqi patient population. By utilizing differential expression analysis between CRC patients and healthy controls, this study seeks to evaluate the efficacy of *miR-205, BCL2,* and *YAP1* as prospective diagnostic and prognostic factors.

Materials and Methods

Patients and control

This case-control study investigated the expression levels of *BCL2*, *YAP1,* and *MiR-205* in blood samples of Iraqi CRC patients. Forty control samples and eighty samples from patients with CRC were collected from Oncology Medical Hospital, Baghdad, Iraq, in the period June 2024 – August 2024. The histological diagnosis of CRC was confirmed in all selected patients with CRC, while control samples were taken from patients indicated for colonoscopy, without inflammatory bowel disease or cancer. Age, sex, body mass index, and chemotherapy history, old chronic disease medications, and cancer family history were also collected.

Inclusion criteria

The study included individuals clinically diagnosed with CRC at a specialized oncology teaching hospital. The control group consisted of age-matched, apparently healthy subjects with no evidence of CRC or other oncological conditions.

Exclusion criteria

To ensure a high-quality study, the current study excluded individuals under 18 years of age from both groups. The control group was further limited to those with no history of cancer and no current thyroid or other major medical conditions.

Blood sample collection

A blood sample of 5 mL was taken from each participant of each group, of which 2 mL was stored in 250 µL Ethylenediaminetetraacetic Acid (EDTA) tubes for the complete blood count (CBC) test. In an Eppendorf tube, 250 µL of blood was mixed with 750 µL TransZol Up (for analysis of cells and to preserve RNA from degradation) to quantify the gene expression of *miR-205, BCL2,* and *YAP1* by quantitative real-time polymerase chain reaction (qRT-PCR). Amount: 2 mL for the serological test.

Demographic and clinical characteristics of patients and Control

The participants' demographic characteristics, including age and Body Mass Index (BMI), were recorded at the time of enrollment. BMI was determined as body weight in kilograms divided by the square of height in meters (kg/m^2). Total White Blood Cell (WBC) counts were determined using an automated hematology analyzer (BC-5150, Mindray company, Darmstadt, Germany), following the manufacturer's instructions.[12] Furthermore, serum concentrations of cytokines, including Interleukin-18 (IL-18) (cat no: SL0980Hu, Sunlong, Hangzhou, China) and Interleukin-22 (IL-22) (cat no: SL0988Hu, Sunlong, Hangzhou, China), were measured using commercially available enzyme-linked immunosorbent assay.[13]

Molecular study

RNA extraction and cDNA synthesis for messenger RNA and miRNA

RNA was isolated from the samples using the TransZol Up Plus RNA Kit (Transgen, China, ER501-10). The concentration and purity of RNA were then assessed using Nanodrop. EasyScript® One-Step cDNA Synthesis and gDNA Removal. A 20 µL reaction volume was performed, and TransStart® top green qPCR Super Mix was utilized as directed by the manufacturer. The expression levels of the *BCL2*, *YAP1*, and *miR-205* genes were assessed using qRT-PCR. The expression of the target gene was confirmed using a qRT-PCR N'-dimethyl-N-[4-[(Z)-(3-methyl-1,3-benzothiazol-2-ylidene) methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine (SYBR) Green dye. Table 1 lists the primer sequences.

The endogenous control (housekeeping gene), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), was selected, owing to its constitutive and stable expression across diverse biological samples and experimental conditions, and the snRNA-U6 levels (housekeeping gene) were produced using the gene primer sequence, amplified, and used to normalize the housekeeping mRNA levels and miRNA. qRT-PCR was conducted using the Qiagen Rotor-Gene real-time PCR system and qPCR soft software (Qiagen company, Hilden, Germany).[18]

Gene expression

Expression analysis of *BCL2*, *YAP1*, and *miR-205* was performed via qRT-PCR. Transcript levels of the target genes were normalized against the housekeeping gene *GAPDH*, while U6 served as the endogenous control for *miR-205* quantification.[19] The reaction was carried out in a final volume of 20µL following the manufacturer's instructions. The reaction mixture consisted of 10µL of SYBR Green Master Mix, 3µL of cDNA, 1µL of forward primer, 1µL of reverse primer, and 5µL of nuclease-free water, according to Ghareeb *et al*.[15] While the thermal profile was performed according to Hasan *et al*.[16] Relative gene expression was calculated using the threshold cycle (Ct) values, and the fold change in expression was calculated using the $2^{-\Delta Ct}$ method.[20]

Primer design targeting miR-205, BCL2, and YAP1 in Iraqi patients with CRC

Specific primers for *BCL2*, *YAP1*, and *miR-205* were implemented using multi-step computation to ensure that the amplification efficiency and specificity were very high. Primer3 was used to produce preliminary primer sets using standardized conditions such as melting temperature (Tm), sequence length, and GC content. In addition, these candidates were refined using deep learning platforms (e.g., DeepPrimer) to estimate binding regions and limit the risk for secondary structures (e.g., hairpins and primer-dimers). The Benchling bioinformatics environment was applied to in silico modeling primer behavior to control for sequence accuracy. Specificity was confirmed by matching the primer to reference human genomic databases to avoid off-target amplification. This coordinated workflow facilitated the choice of optimal binding sites and the reliable quantification of gene expression levels in CRC samples.[21–24]

Statistical analysis

Relative expression levels of *miR-205, BCL2*, and *YAP1* were quantified using the $2^{\wedge-\Delta Ct}$ method, with values normalized against the internal control (*snRNA-U6* for miRNA and *GAPDH* for mRNA), then fold change in gene expression, with the control group serving as the calibrator (value:1.00), was calculated. Data were analyzed using GraphPad Prism (version 9.0; GraphPad Software Inc., San Diego, CA, USA) and SPSS (version 29.0; IBM Corp., Armonk, NY, USA). The distribution of data was checked for normality through the Shapiro-Wilk and Kolmogorov-Smirnov tests. For CRC patients vs. healthy controls, the student's t-test was used for normally distributed data, and results were reported as the mean ± its Standard Deviation (SD). The diagnostic value of the respective markers was assessed by the Receiver Operating Characteristic (ROC) curve, and Area under the Curve (AUC), sensitivity, and specificity were calculated. In addition, the associations of the expression levels of the analyzed variables were evaluated with Pearson's correlation coefficient. Statistical significance of all tests was obtained at $p < 0.05$.[13]

Results

Demographic and clinical characteristics of patients and Control

Table 2 shows the demographic and clinical characteristics of patients and control. The mean age of the control group was 58.53 years, while that of CRC patients was 56.791 years, with no significant difference between the two groups ($p = 0.7$). Similarly, Body Mass Index (BMI) values (kg/m^2) showed no significant variation, with patients averaging 26.88 and Control 26.80 ($p = 0.9$). Gender distribution also did not differ significantly: men accounted for 37.5% of the patient group and 57.5% of the control group, while women were 62.5% and 42.5%, respectively ($p = 0.2$). Mean WBC levels showed a slight reduction in patients (7.05) compared to Control (7.98), but this difference was not statistically significant ($p = 0.09$). Additionally, cytokine evaluation revealed no significant differences in IL-18 ($p = 0.7$) or IL-22 ($p = 0.6$) levels between patients and healthy individuals.

Molecular study results

Purity and concentration of RNA

The extracted RNA concentration and purity were quantified by NanoDrop One^C spectrophotometer (Thermo Fisher Scientific, USA). RNA concentrations across samples ranged from 27 to 39 ng/µL and were analyzed for the absence of protein and organic contaminant interference through optical density (OD) absorbance ratios. The A260/A280 ratio in all of the samples ranged from 2.00 to 2.02, demonstrating high quality RNA applicable for downstream molecular applications.

Validation of the reference gene in study groups

Gene expression levels were normalized using the housekeeping genes *GAPDH* (for mRNA) and *snRNA-U6* (for miRNA). The quantification was performed using the ΔCt and relative expression ($2^{-\Delta Ct}$) method to evaluate the transcript abundance in each group. Amplification curve and disassociation curve of *GAPDH* and *snRNA-U6* were presented in Figure 1a and 1b.

The comparison of mean Ct values between CRC patients and healthy control showed no statistically significant differences ($p > 0.05$). For *GAPDH*, the mean Ct ± standard deviation (SD) values were 14.26 ± 3.37 in control and 14.59 ± 3.76 in CRC patients, indicating comparable

expression between groups. Similarly, *snRNA-U6* exhibited 12.38 ± 2.52 in control and 12.35 ± 3.18 in CRC patients, also demonstrating no significant difference in expression levels.

Quantification of miR-205, BCL2 and YAP1 expression

Figure 2 (a, b, and c) showed the amplification plots and dissociation curves for *miR-205*, *BCL2* and *YAP1*, respectively. Expression levels were quantified using $2^{\wedge- \Delta Ct}$ method, as summarized in Table 1S in the supplementary file. Overall, gene expression levels were significantly altered in CRC patients, and Figure 3 illustrates the fold expression level of the three molecular parameters among both study groups.

The mean Ct value for *GAPDH* was 14.26 in control while 14.59 in CRC patients. Similarly, the *snRNA-U6* mean Ct for both control and CRC patients were 12.38 ± 2.52 and 12.35 ± 3.18 respectively, verifying a consistent reference gene expression. Fold change of miR-205 was significant downregulation in CRC patients (Fold: 0.33, p = 0.004) while *BCL2* expression was elevated 1.92-fold. Subsequently, *YAP1* expression increased 1.44-fold in CRC patients, as shown in Table 1S in the supplementary file.

The receiver operating characteristic curve

ROC curve analysis was performed to assess the diagnostic performance of *miR-205*, *BCL2*, and *YAP1* in distinguishing CRC patients from control. As demonstrate in Table 3 and Figure 1S in the supplementary file, all three parameters demonstrated measurable separation between sensitivity and specificity.

The ROC findings revealed that miR-205 had an AUC of 0.700 (p=0.05) with a best cutoff value of 0.2275, resulting in 91% sensitivity and 58% specificity. *BCL2* showed the highest diagnostic accuracy with an AUC of 0.880 (p= 0.001), a cutoff value of 1.4677, and balanced sensitivity and specificity with value 83%. YAP1 demonstrated an AUC of 0.736 (p= 0.02) with best cutoff of 1.0977 yielding 79% sensitivity and 75% specificity.

Pearson correlation coefficient factor

Pearson correlation analysis was conducted to evaluated the statistical associations between *miR-205, BCL2,* and *YAP1* mRNA expression levels in CRC patients. The current findings summarized in Table 2S in the supplementary file, reveal varying degrees of relatedness among these factors. A moderate positive correlation was observed between *miR-205* and *BCL2* ($r = 0.365$, $p = 0.029$). While this statistical association is significant, it does not confirm a direct regulatory mechanism, as the observed trend may result from shared upstream regulators or indirect signaling crosstalk within the tumor microenvironment. In contrast. The association between *miR-205* and *YAP1* was weak and did not reach statistical significance ($r = 0.236$, $p = 0.166$). However, a statistically significant positive correlation was identified between *BCL2* and *YAP1* ($r = 0.556$, $p < 0.001$).

Discussion

This study aimed to investigate the expression profiles of *miR-205*, *BCL2*, and *YAP1* in Iraqi CRC patients to identify potential diagnostic and prognostic parameters. By comparing these molecular indicators between patients and healthy Control, we explored their roles in tumor suppression, apoptosis regulation, and oncogenic signaling. Understanding these interactions may provide insight into CRC progression and support the development of targeted diagnostic and therapeutic strategies. Consistent with previous investigations, our findings demonstrate that CRC patients and healthy Control were well-matched for age and BMI,[25] ensuring that demographic variables did not confuse outcomes of the current study. Furthermore, the comparable gender distribution among groups emphasizes the balanced nature of the study design, ensuring that sex-linked physiological factors do not bias the molecular outcomes. The absence of significant disparities in WBC counts indicates that baseline systemic inflammatory status was uniform between CRC patients and healthy Control.[26] Likewise, the lack of significant variation in IL-18 and IL-22 levels in accordance with previous studies.[27,28] suggests that these specific cytokines may not serve as discriminative indicators for CRC. This observation aligns with previous investigations, which indicate that systemic cytokine shifts can be highly dependent on the clinical stage of the disease or specific regional genetic backgrounds.[29]

Housekeeping genes are commonly employed in molecular studies as an internal Control because they are presumed to maintain stable expression across different tissues and experimental

conditions.[30] *GAPDH* and U6, widely recognized for their consistent transcriptional activity,[31] exhibited similar Ct values in both CRC patients and healthy Control in this study, confirming their suitability as reference genes for normalization. The stability of these reference genes ensures the reliability of downstream comparative expression analyses. The observed downregulation of *miR-205* (0.33-fold) supports its proposed tumor-suppressor function.[30] Reduced *miR-205* expression may facilitate tumor proliferation and invasion, consistent with previous reports linking low *miR-205* levels to aggressive CRC behavior and poor prognosis.[15,32] In contrast, BCL2 was upregulated 1.92-fold, suggesting that CRC cells may evade apoptosis by overexpressing this anti-apoptotic protein.[33] Consistent with previous investigations into Hippo pathway dysregulation, we observed a 1.44-fold upregulation of *YAP1*. This increase underscores its contribution to colorectal carcinogenesis and metastasis, likely through synergistic cross-talk with the Wnt/β-catenin pathway, which has been shown to drive therapeutic resistance.[34,35]

ROC curve analysis is widely applied in clinical epidemiology to assess the diagnostic accuracy of medical tests that classify individuals into "diseased" versus "non-diseased" groups.[4,41] The AUC provides a robust measure of test validity by combining sensitivity and specificity into a single performance index.[36,37] The diagnostic efficacy of the studied parameters was validated via ROC analysis, where *BCL2* has a high AUC (0.88) and balanced sensitivity/specificity (83%), suggesting a strong capability for discriminating CRC patients from healthy Control, which agrees with the previous study.[38] While *YAP1* showed more modest diagnostic parameters (AUC = 0.74), its contribution to the molecular profile of the disease with a significant p-value (0.02), particularly when considering its role in Hippo pathway dysregulation,[39] while *miR-205* exhibited high sensitivity (91%) but lower specificity (58%), suggesting its value as a screening indicator when combined with other biomarkers.[40] The integration of these markers into a multi-biomarker panel may overcome individual limitations and enhance diagnostic accuracy.

Correlation analysis showed that *miR-205* was moderately positively associated with *BCL2* ($r = 0.365$; $p = 0.029$). Yet, this observed significant association did not represent that *miR-205* regulates *BCL2*, but that the upregulation of *BCL2* is indeed also a compensatory mechanism alongside the pro-apoptotic effect of *miR-205*.[41,42] A weak association between *miR-205* and *YAP1* was detected but not significant ($r = 0.24$, $p = 0.2$). Given this lack of association, their co-

expression in CRC may be instead secondary to indirectly regulated processes or secondary oncogenic pathways and not a direct miRNA-target interaction.[43,44]

Importantly, this apparent positive association between *BCL2* and *YAP1* ($r = 0.556$, $p < 0.001$) could suggest a functional engagement of apoptosis and Hippo-YAP1 signaling for the progression of CRC and therapeutic resistance. Our results imply that a signaling crosstalk such as this has the potential to enhance therapeutic sensitivity in CRC, and support a need for further data-driven personalized and multi-targeted therapeutic strategies.[45] Nevertheless, our investigation is limited, as the data for *miR-205*, *BCL2,* and *YAP1* as CRC predictive signs in this study are strong. The sample size was relatively small, and three molecular parameters were studied, impairing generalizability. Larger cohorts with additional validation involving molecular or clinical-related factors would have to be validated to confirm these findings and complete the understanding of the mechanisms driving the progression of CRC.

Despite the significant findings regarding *miR-205, BCL2*, and *YAP1*, several limitations should be acknowledged in the present study. One important limitation was the lack of comprehensive clinicopathological stratification, including tumor stage, histopathological grade, and metastatic status. Consequently, the association between the expression patterns of these biomarkers and disease progression could not be fully evaluated. Therefore, future studies involving larger cohorts and more detailed clinical data are recommended to further validate the diagnostic and prognostic significance of these biomarkers across different stages of colorectal cancer.

Conclusions

In this study, *miR-205, BCL2,* and *YAP1* were shown to have some diagnostic value as diagnostic indicators in CRC patients, with *BCL2* possessing the highest capacity by means of the ROC curve analysis. The downregulation of *miR-205* and upregulation of *BCL2* and *YAP1* that were found in the study confirm *miR-205*-mediated tumor inhibition potential in CRC, while *BCL2* and *YAP1* may also perform as oncogenes. Correlation profiles indicate that decreased *miR-205* may serve as a *BCL2* factor to block apoptosis, associated with *BCL2* and *YAP1* activation, indicating interaction between apoptosis inhibition and tumor proliferation pathways.

The lack of correlation between *miR-205* and *YAP1* suggests separate influences on CRC's progression. The important limitations of this study are limited by a relatively small number of patients involved, and hence, some potential limitations in the generalizability of results, and only focusing on three biomarkers without consideration of other pertinent molecular or clinical features. Larger and more heterogeneous populations should be included in future research, the analysis of biomarkers should include more diverse populations and biological and clinical indicators to develop a full diagnostic profile, and make targeted therapeutic and diagnostic algorithms for CRC in particular among high-risk subgroups possible.

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Table 1. Primers used in the study.

Primer	Sequence from 5'–3' direction		Annealing Temperature (°C)	Ref.
	Forward	**Reverse**		
BCL2	CTTCAGGCAAAACGTCGAAT	GGTGCTTGGCAATTAGTGGT	60	*
YAP1	CACAGCTCAGCATCTTCGAC	TGTGACGTTCATCTGGGACA	60	*
GAPDH	GGCCTCCAAGGAGTAAGACC	AGGGGTCTACATGGCAACTG	58	[14]
miRNA205	TCCTTCATTCCACCGGAGTCTGT		66	[15]
miRU6 F.	AGAGAAGATTAGCATGGCCCCT		58	[16]
universal R. transcription	CAGGTCCAGTTTTTTTTTTTTTTVN		-	[17]
miRNA-universe R.	GCGAGCACAGAATTAATACGAC		-	[17]

GAPDH, Glyceraldehyde-3-phosphat dehydrogenase, *BCL2*, B-cell lymphoma 2, *YAP1*, Yes-Associated Protein 1. * Means designed according to current study

Table 2. Demographic and clinical characteristics of patients and control.

Characteristics	Patients (no.80)	Control (no.40)
Age (Mean ± SD)	56.791 ±9.32	58.533 ±9.409
p value	**0.7 NS**	

BMI (Mean ± SD)		26.877± 6.49	26.800± 2.90
p value		**0.9 NS**	
Gender	Women (no)	50 (62.5%)	17 (42.5%)
	Men (no)	30 (37.5%)	23 (57.5%)
p value		**0.2 NS**	
WBC (Mean ± SD)		7.052± 2.32	7.98±2.79
p value		**0.09 NS**	
IL-18 (Mean ± SD)		17.229±3.30	18.610±4.78
p value		**0.7 NS**	
IL-22 (Mean ± SD)		22.525±4.74	23.283±4.32
p value		**0.6 NS**	

* Numerical data were expressed as Mean ± Standard Deviation (SD) and analyzed using the Independent T-test. Categorical data were presented as frequencies and percentages and analyzed using the Chi-square test. NS: Non-Significant ($p > 0.05$). WBC, White Blood Cell; BMI, Body Mass Index; IL-18, Interleukin-18; IL-22, Interleukin-22.

Table 3, Receiver operating characteristic curve data of the studied gene.

Parameters	**AUC**	**p value**	**The best cut-off**	**Sensitivity %**	**Specificity%**
MiR-205	0.700	0.05	0.228	91	58
BCL2	0.880	0.001	1.468	83	83
YAP1	0.736	0.02	1.098	79	75

*AUC: Area under the curve

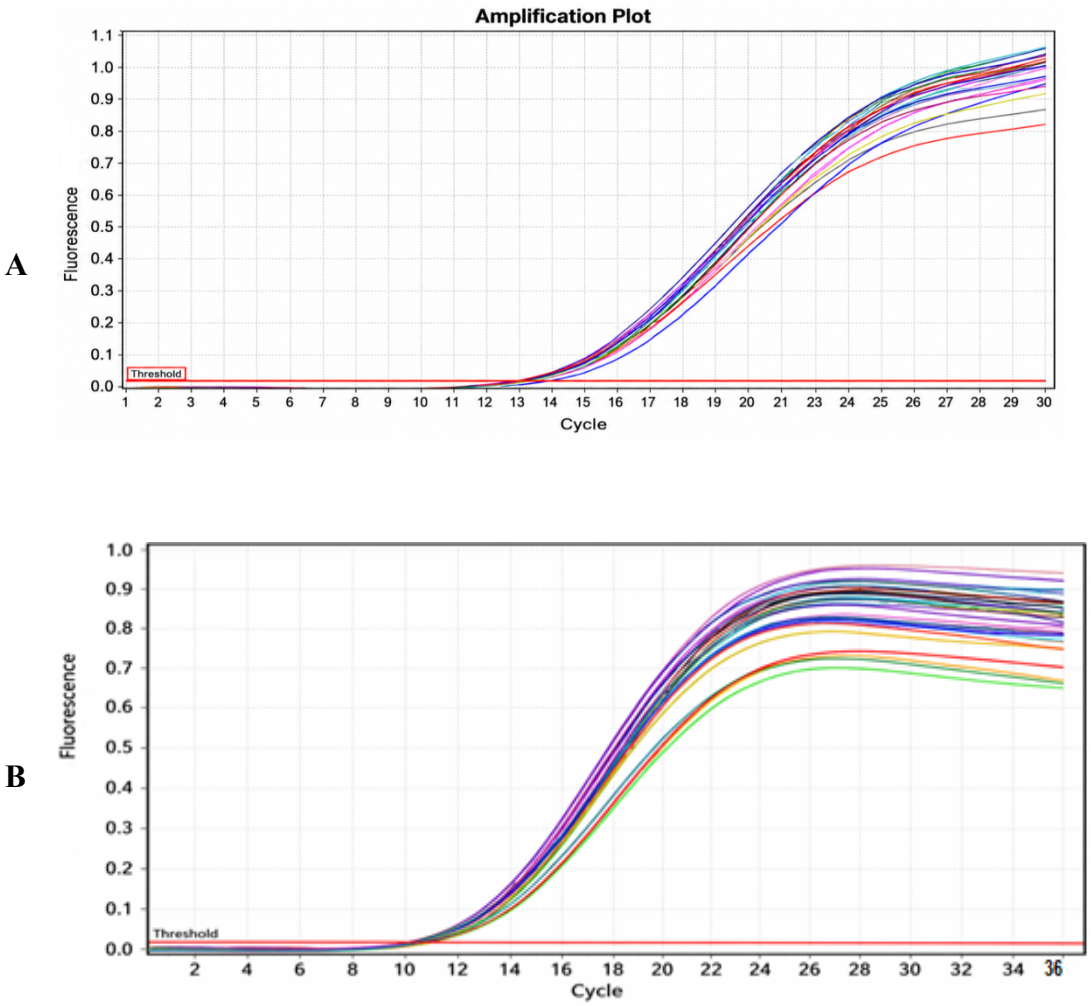
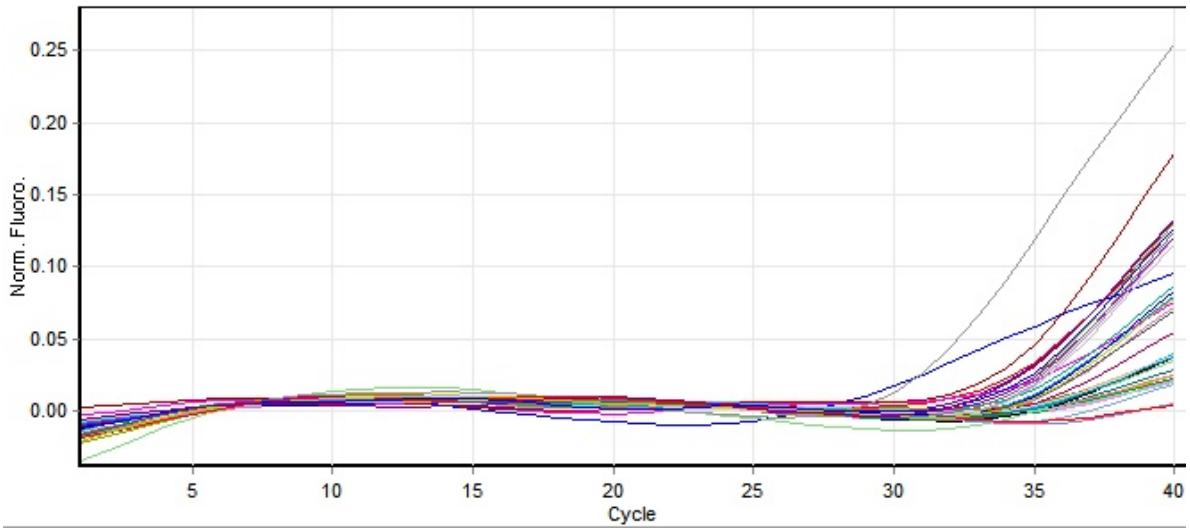


Figure 1. Amplification plots of qRT-PCR analysis showing (a) glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and (b) *snRNA-U6* expression across the study groups. Each curve represents an individual sample analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). The y-axis indicates Normalized Fluorescence, representing fluorescence intensity normalized to the background signal. Images were obtained directly using the Qiagen Rotor-Gene Q 6000 qRT-PCR system.

A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0.15
0.10
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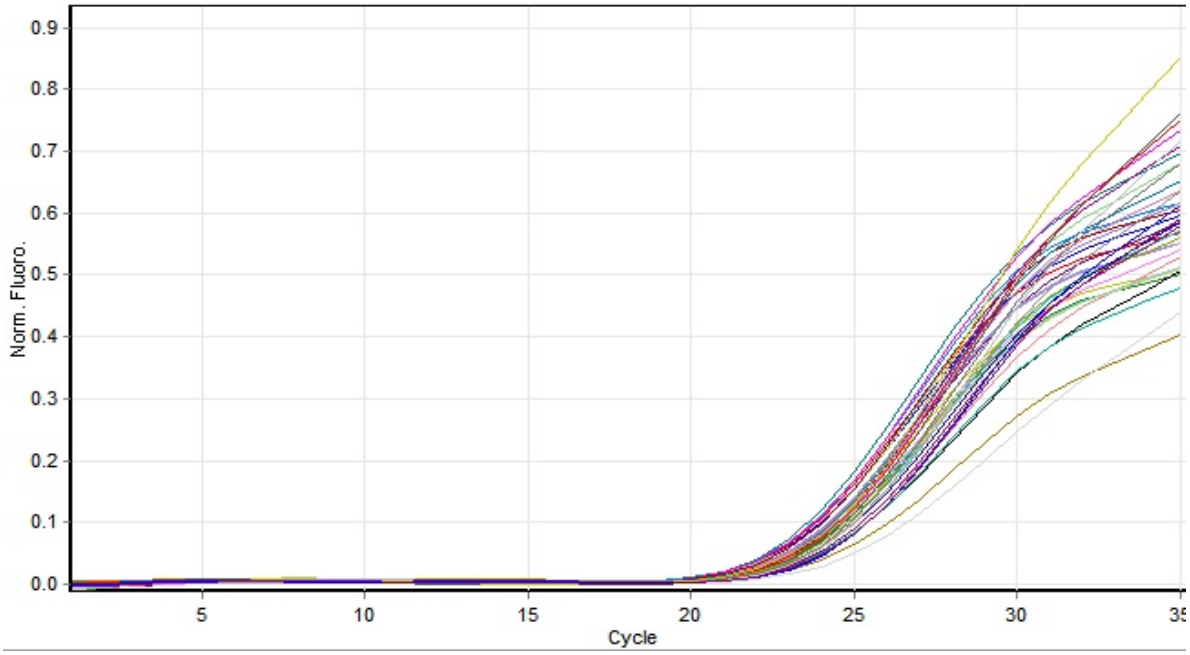
0.00
5
10
15
20
25
30
35
40
Cycle

B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5
10
15
20
25
30
Cycle

C

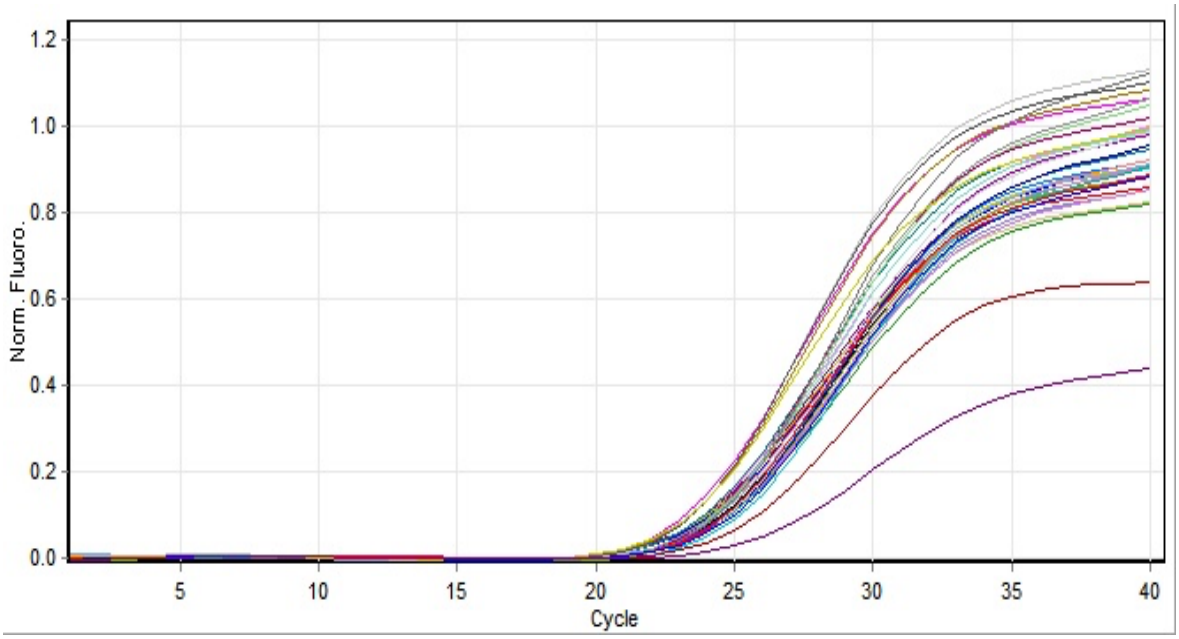

Figure 2. Amplification plots of qRT-PCR analysis showing (a) *miR-205*, (b) *BCL2*, and (c) *YAP1* gene expression across the study groups. Each curve represents an individual sample analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). The y-axis indicates Normalized Fluorescence (Norm. Fluoro.), which represents fluorescence intensity adjusted relative to background signal. The images were obtained directly using the Qiagen Rotor-Gene Q 6000 qRT-PCR system.

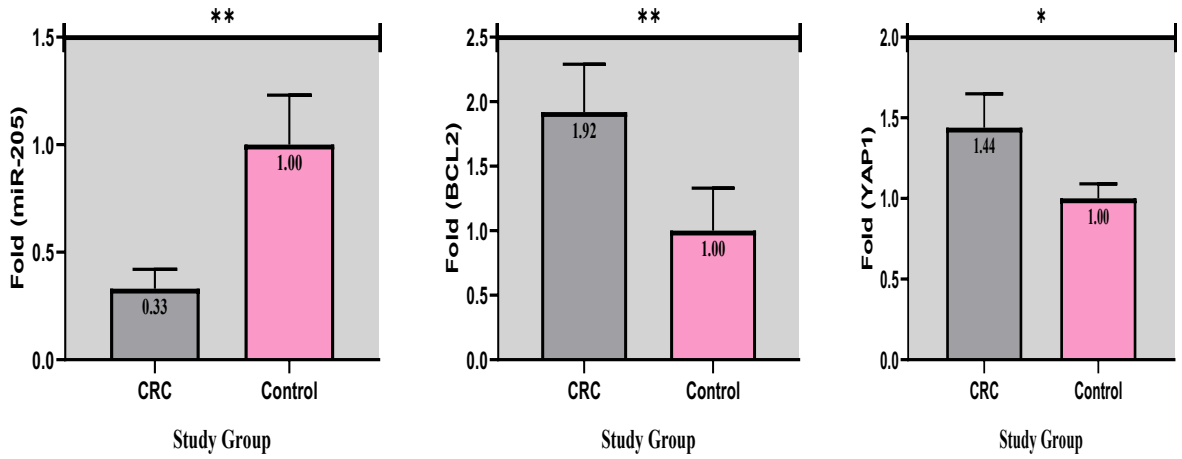

Figure 3. Differences between the Colorectal Cancer (CRC) patients and control according to a) *miR-205*, b) *BCL2*, and c) *YAP1* gene expression. * Significant. ** highly significant.

Online supplementary materials

Table 1S. Expression levels of *miR-205*, *BCL2* and *YAP1* determined in control and patient groups using the 2−ΔCt method.

Table 2S. Pearson correlation coefficient factor.

Figure 1S. Receiver Operating Characteristic (ROC) curve of (a) *MicroRNA-205*, (b) *BCL2*, and (c) *YAP1*.

Contributions

Asmaa Mahmood Salman Al-Obaidi contributed to the methodology, investigation, and preparation of the original draft. Alaa H. Younus was responsible for data collection, project administration, and research supervision. Ghanyia Jasim Shanyoor contributed to the data curation, methodology, and manuscript writing. Sarab Hussain Khaleel contributed to visualization and critically reviewed and edited the manuscript. Mohanad K. Aneed Al Saedi conceived the study,

performed the formal analysis and data interpretation, supervised the research, and critically reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Ethical Approval

The Institutional Review Board (IRB) of Oncology Medical Hospital, Baghdad, Iraq, approved the study protocol in the period June 2024 - August 2024 with reference no. (51895). Informed written consent was obtained from all participants before blood sampling.

Financial support and sponsorship

No specific grant from a public, private, or nonprofit funding organization was obtained for this study.

Conflicts of interest

There is no conflict of interest.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.