RESEARCH ARTICLE

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Gene Expression of *MicroRNA-205, FGF2* and *CARMA3* in Colorectal Cancer in Iraqi Patients

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Abstract

Background: Colorectal cancer (CRC) is a growing global health concern. This study focuses on evaluating the gene expression of *FGF2, CARMA3*, and *MicroRNA205 (miR205)* as potential blood-based biomarkers for early CRC diagnosis by distinguishing patients from healthy controls. **Materials and Methods:** Blood samples from 80 colorectal cancer (CRC) patients and 40 healthy controls were analyzed using qRT- PCR to measure target gene expression. ROC curve analysis was performed to evaluate diagnostic accuracy, including sensitivity, specificity, and Area Under the Curve (AUC) values. **Results:** *MicroRNA-205* revealed downregulation in CRC patients, with an AUC of 0.7, sensitivity of 91%, and specificity of 58%. AUC values for Fibroblast Growth Factor 2 *(FGF2)* and Caspase Recruitment Domain Family Member 3 *(CARMA3)* were 0.74 and 0.77, respectively, indicating differential expression. All markers displayed modest specificity, but CARMA3 showed the highest diagnostic accuracy with 95% sensitivity. Gene expression fold change for miR-205 showed downregulation 0.3, FGF2 also showed downregulation 0.4 and CARMA3 showed slight upregulation 1.2. **Conclusion:** The results suggest that *miR-205*, *FGF2,* and *CARMA3* may serve as potential biomarkers for the identification of colorectal cancer (CRC), particularly when used in multi-marker panels to improve diagnostic accuracy. Their clinical utility should be confirmed through additional validation in larger cohorts.

Keywords: *miR-205- FGF2- CARMA3-* CRC- Gene expression- ROC Curve.

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Introduction

Colorectal cancer (CRC) ranks as the second most prevalent malignancy among adult females and the third most frequent among adult males. Furthermore, CRC stands as the fourth principal contributor to cancer-related mortality, representing 9.2% of global deaths [1].

(CRC) is one of the most prevalent cancers globally, also associated with high morbidity and mortality rates. It the second leading cause of cancer death after lung cancer and the third most easily diagnosed cancer [2]. CRC is amongst the most widespread malignancies and increase in incidence has already been detected in Iraq which is alarming on a public health level [3]. In addition to its well-established genetic basis, CRC develops through a complex interplay of environmental and epigenetic factors, reflecting a multifactorial pathophysiological process [4].

Gene expression profiling is a powerful tool for understanding cancer biology and identifying potential biomarkers for diagnosis, prognosis, and treatment personalization [5]. Among the various molecular regulators, microRNAs, with their new capacity to modulate gene expression at post-transcriptional levels, have attracted special interest [6]. Dysregulation of particular miRNAs has been associated with many cancers, including colorectal cancer [7].

MicroRNA205 (miR205) is a small non-coding RNA that regulates biological functions like differentiation, apoptosis and proliferation [8]. The gene/protein exhibits dual functions as both an oncogene and a tumor suppressor depending on the tissue context, representing a unique duality in cancer biology [9]. However, its specific role and expression profile in colorectal cancer (CRC), particularly among patients from Iraq, remain poorly understood [10].

Fibroblast growth factor 2 (*FGF2*) is a potent modulator of angiogenesis, tissue repair and cell proliferation [11]. Many tumors overexpress Fibroblast Growth Factor 2, and high levels of this growth factor in the tumor milieu are often associated with enhanced tumor growth, invasion, and metastasis [12]. This knowledge regarding the expression of *FGF2* in colorectal cancer would give an indication on how it might play a role in

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tumor development [13].

Caspase Recruitment Domain Family Member 3 (*CARMA3*) A scaffold protein that regulates activation of the nuclear factor kappa B (NF-κB) signaling pathway, a critical pathway for tumorigenesis and inflammation [14]. Cellular activation of NF-κB is associated with CRC development; therefore *CARMA3* represents a potential molecular target for cancer therapy [15].

The aim of this study to investigate the level of expression *miR-205, FGF2* and *CARMA3* gene in colorectal cancer as well that expressed in Iraqi patients. The next step will be to assess their potential in playing a role in CRC progression and examining their differential expression, to aid as new diagnostic/prognostic markers. Knowledge of these molecular mechanisms might help in the development of targeted therapies and enhancement of clinical outcomes for CRC patients in Iraqi population.

Materials and Methods

A case-control study examined the expression level of *FGF2*, *CARMA3*, is an abbreviation of caspase-recruitment domain membrane-associated guanylate kinase protein 1CAR a3, and MicroRNA205 in blood samples of Iraqi colorectal cancer patients. Blood samples were collected between June and August 2024 from 80 colorectal cancer patients and 40 control subjects at Baghdad Medical Complex, Baghdad, Iraq. The histological diagnosis of colorectal cancer was established in all colorectal cancer selected patients while control samples were drawn from patients indicated for colonoscopy and found having no inflammatory bowel disease or cancer. Personal information, including age, sex, BMI and chemotherapy history, drugs for chronic illnesses, and family history of cancer were also recorded.

The study included patients clinically diagnosed with colorectal cancer at an oncology teaching hospital, as well as a healthy control group consisting of individuals who were apparently healthy, free from colorectal cancer or any other form of cancer, and without thyroid or other diseases. Pediatric patients and any controls who were children were excluded. Additionally, healthy controls with any form of cancer or a family history of cancer were excluded from the study.

Blood sample collection

A sample of blood Each member of each group had 5 mL of blood drawn, and 3 mL of the blood was stored in 250 µL ethylenediaminetetraacetic acid tubes for CBC test. To quantify the gene expression of MicroRNA205, *FGF2*, and *CARMA3* by quantitative real time PCR (qRT PCR), 250 µL of blood was added to 750 µL TransZol Up in an Eppendorf tube. Additionally, 2 mL was used for the enzyme linked immunosorbent assay (ELISA) for IL18 and IL22.

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 determined using Nanodrop. EasyScript® One-Step cDNA Synthesis and gDNA Removal. A 20 µl reaction volume was employed, and TransStart® top green qPCR Super Mix was utilized as directed by the manufacturer. qRT-PCR The expression levels of the *FGF2*, *CARMA3*, and *MicroRNA-205* genes were assessed using qRT-PCR. The expression of the target gene was verified using a quantitative real-time qRT PCR SYBR Green assay. Table 1 lists the primer sequences. The endogenous control (housekeeping gene), glyceraldehyde 3 phosphate dehydrogenase (*GAPDH*), and the miRNA levels of U6 (housekeeping gene) were produced using the gene primer sequence, amplified, and utilized to normalize the housekeeping mRNA levels and miRNA [16]. QRT PCR was conducted using the Qiagen Rotor Gene real time PCR system and qPCR soft software. Using the elements of the TransStart® Top Green qPCR SuperMix Kits, the fold change and gene expression levels were measured using the cycle threshold (Ct).

Molecular analysis revealed that transcriptome total RNA was successfully extracted from each blood sample.

Gene expression

In the current study, gene expression was evaluated using RT-qPCR, a fluorescent dye that can detect any kind of double-strand DNA, including cDNA, and a Ct value that indicates amplification was noted. Greater amounts of the target were indicated by a lower Ct value, and the reverse was also true. High gene expression is indicated by a low Ct value, while low gene expression is indicated by a high value.

Relative gene expression measurement using quantitative Real time reverse transcription quantitative polymerase chain reaction

To confirm the target gene's expression, RT-PCR was used to determine the levels of expression for the housekeeping gene *GAPDH* and *FGF2*, and *CARMA3* and the housekeeping gene (U6) for *MicroRNA-205* [17].

Gene expression calculation

The ratio that depends on the calibrator value, the mean ΔCt of the patients, and the mean ΔCt of the control is known as the degree of gene expression fold. Without the housekeeping gene values, none of the computations could be completed [18].

Statistical Analysis

Gene expression levels were calculated using the Livak $2^{-\Delta Ct}$ method. Data were analyzed using GraphPad 9 and SPSS 29. Student's t-test or Mann–Whitney U test was applied to clinical and biochemical parameters. ROC curve analysis assessed diagnostic performance, and Pearson's correlation evaluated relationships among biomarkers. A p-value < 0.05 was considered significant.

Results

Validation of reference gene in study groups

Gene expression was normalized to *GAPDH* and U6 using the ΔCt and $2^{\Delta Ct}$ methods (Figure 1a, 1b). For

Table 1. The Primers Used in the Study

Primer	Sequence from 5'–3' direction		Annealing Temperature (°C)
	Forward	Reverse	
FGF2	GGTGAAACCCCGTCTCTACA	ACCTTGACCTCTCAGCCTCA	60 °C
CARMA3	GCCTTCCTAGACCCTTGGAC	GCAGCAAGTAGAGGGGAGTG	60 °C
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC	
miRNA205	TCCTTCATTCCACCGGAGTCTGT		66 °C
miRU6 F.	AGAGAAGATTAGCATGGCCCCT		-
universal R. transcription	CAGGTCCAGTTTTTTTTTTTTTTTVN		-
miRNA-universe R.	GCGAGCACAGAATTAATACGAC		-

GAPDH, Glyceraldehyde 3 phosphate dehydrogenase, *FGF2,* fibroblast growth factor 2, *CARMA3,* Caspase Recruitment Domain Family Member 3

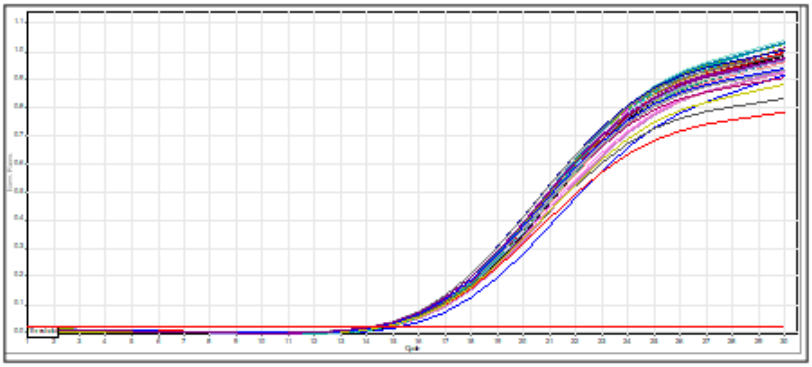
GAPDH the mean Ct ± SD was 14.26 ± 3.37 in controls and 14.58 ± 3.76 in patients. Similarly, for U6, the mean Ct ± SD was 12.38 ± 2.52 in controls and 12.35 ± 3.18 in patients. Mean Ct values did not differ significantly between control and CRC groups in *GAPDH* and U6 (Tables 3, 4, and 5; $p > 0.05$).

Real time polymerase chain reaction quantification of MicroRNA-205, FGF2 and CARMA3 expression

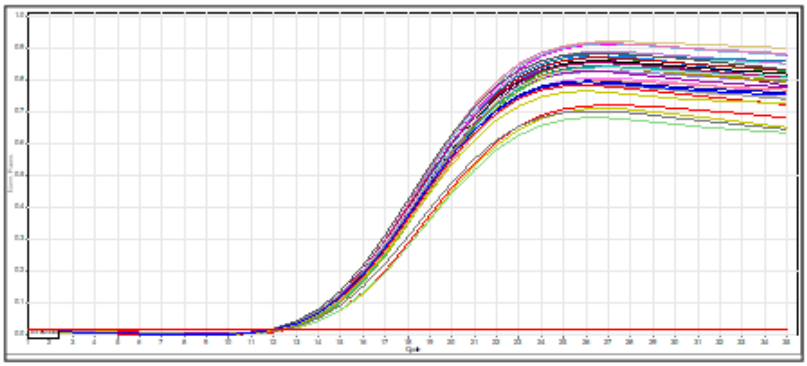
The amplification plots and dissociation curves for *miR-205*, *FGF2*, and *CARMA3* are shown in Figure 2a, 2b, and 2c. Using the $2^{-\Delta Ct}$ method, the relative expression levels of these genes were calculated, and fold changes were determined to compare CRC patients with healthy controls (Tables 2, 3, 4,). A summary of these expression profiles is illustrated in Figure 3. For miR205, the fold change was approximately 0.33, indicating a significant downregulation in CRC patients- roughly three-fold lower expression compared to healthy controls (Table 2). For *FGF2*, the fold change was 0.42, reflecting more than a 50% reduction in expression levels in CRC patients compared to controls (Table 3). In contrast, *CARMA3* exhibited a slight upregulation, with a fold change of 1.20 (Table 4).

AGE and BMI

The control group's mean age was 58.533 years, whereas the patients' mean age was 56.791 years. There was no statistically significant difference in age between the two groups ($p = 0.7$). Similarly, there was no significant difference ($p = 0.9$) in the Body Mass Index (BMI) between the patients (26.877) and controls (26.800) (Table 5).



(a)



(b)

Figure 1. (a) Glyceraldehyde3phosphate dehydrogenase (b) U6 gene every study group was represented in the amplification plots by quantitative polymerase chain reaction (qPCR) samples. The Qiagen Rotor-Gene Q 6000 qPCR machine was used to take the picture directly

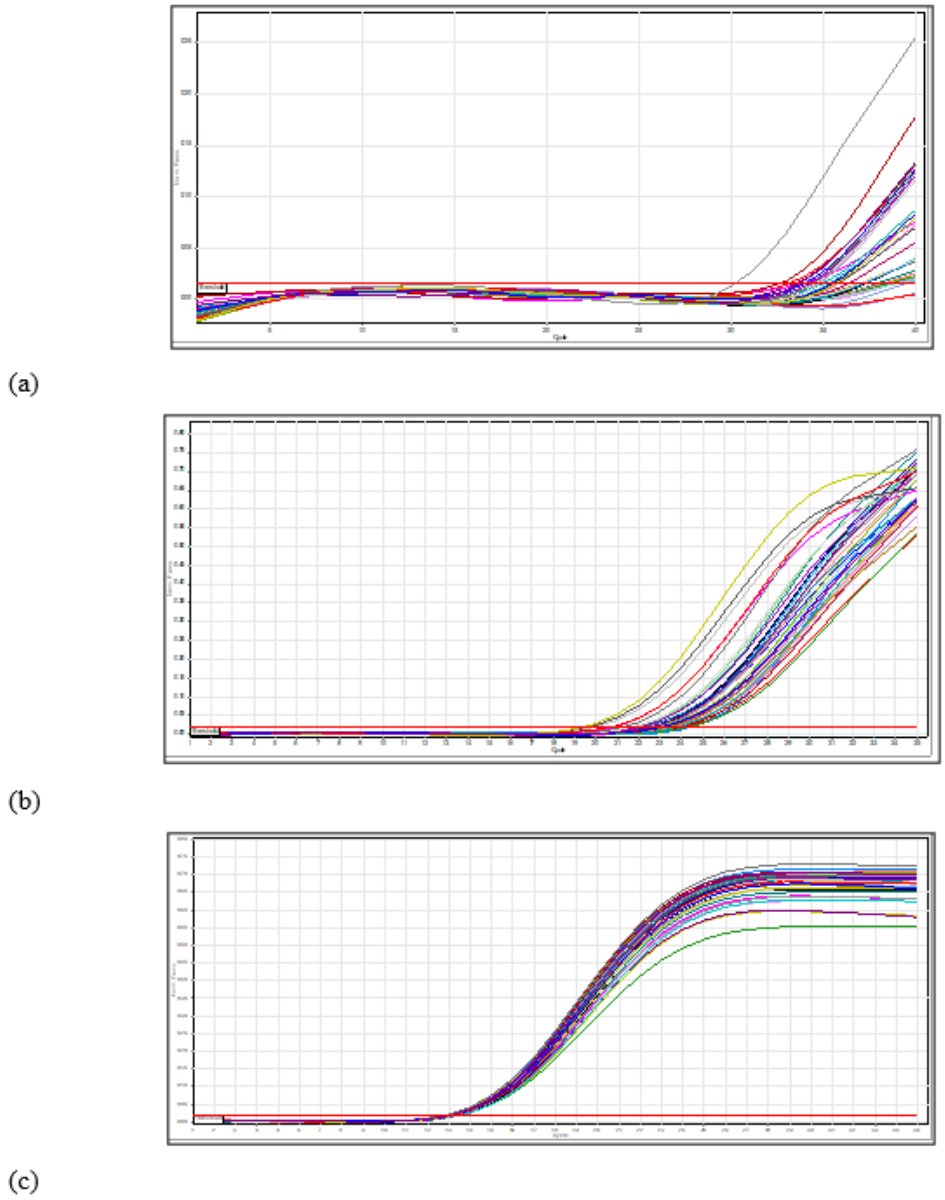


Figure 2. (a) *miRNA-205* (b) *FGF2* (c) *CARMA3* gene. every study group was represented in the amplification plots by quantitative polymerase chain reaction (qPCR) samples. The Qiagen Rotor-Gene Q 6000 qPCR machine was used to take the picture directly.

Distribution of Gender

Males made up a larger percentage of the patient group (62.5%) than the control group (41.7%), though this difference was not statistically significant (p = 0.2) (Table 5).

WBCs

Patients showed slightly lower mean WBC counts (7.052) than controls (7.98), but the difference was not statistically significant (p = 0.09) (Table 5).

Cytokines IL18 and IL22

The levels of IL18 and IL22, two cytokines associated with immune regulation and inflammation, were comparable between the patient and control groups, showing no significant differences (p = 0.7 for IL18 and p = 0.6 for IL22) (Table 5).

The receiver operating characteristic curve

ROC curves showed that *CARMA3* had the highest AUC (0.77) with a sensitivity of 95% but specificity of

Table 2. Using the $2^{-\Delta Ct}$ Method, the Expression Level of MiRNA was Determined in Control and Patient Groups

Groups	Means Ct of Mi205	Means Ct of U6	ΔCt (Means Ct of Mi205)	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Patients	36.19583	12.35083	23.845	0.0000001	0.0000001/0.0000002	0.33
Control	34.68416	12.3858	22.2983	0.0000002	0.0000002/0.0000002	1

Relative gene expression was determined using the Livak $2^{-\Delta Ct}$ comparative method, with U6 as the reference gene for miRNA (18).

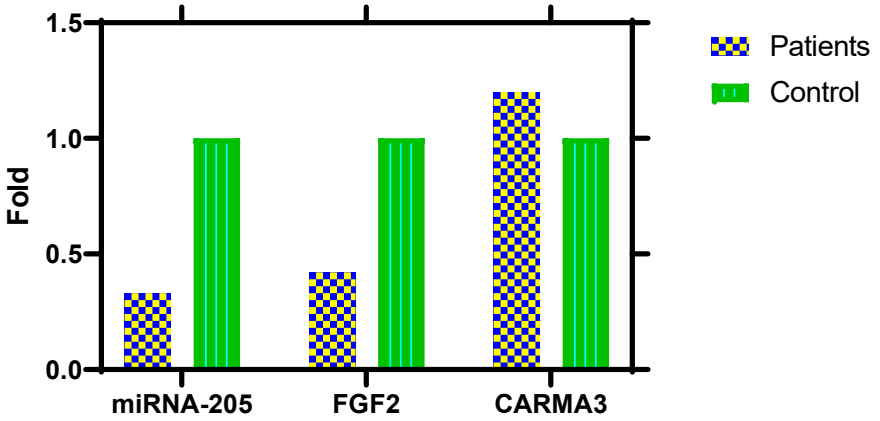


Figure 3. The Differences between the CRC Patient and Control According to *MicroRNA-205, FGF2* and *CARMA3* Gene Expression.

Table 3. Using the $2^{-\Delta Ct}$ Method, the Expression Level of *FGF2* was Determined in Control and Patient Groups

Groups	Means Ct of *FGF2*	Means Ct of *GAPDH*	ΔCt (Means Ct of *FGF2*)	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Patients	23.1033	14.5875	8.5158	0.0027	0.0027/0.0064	0.42
Control	21.5458	14.26	7.2858	0.0064	0.0064/0.0064	1

Relative gene expression was determined using the Livak $2^{-\Delta Ct}$ comparative method, with U6 as the reference gene for miRNA (18).

50%. *FGF2* had an AUC of 0.74 and miR205 0.7, with high sensitivity (91%) but modest specificity (50-58%) (Table 6; Figure 4).

Pearson Correlation

Pearson analysis showed a moderate positive correlation between miR205 and *CARMA3* (r = 0.465, p =

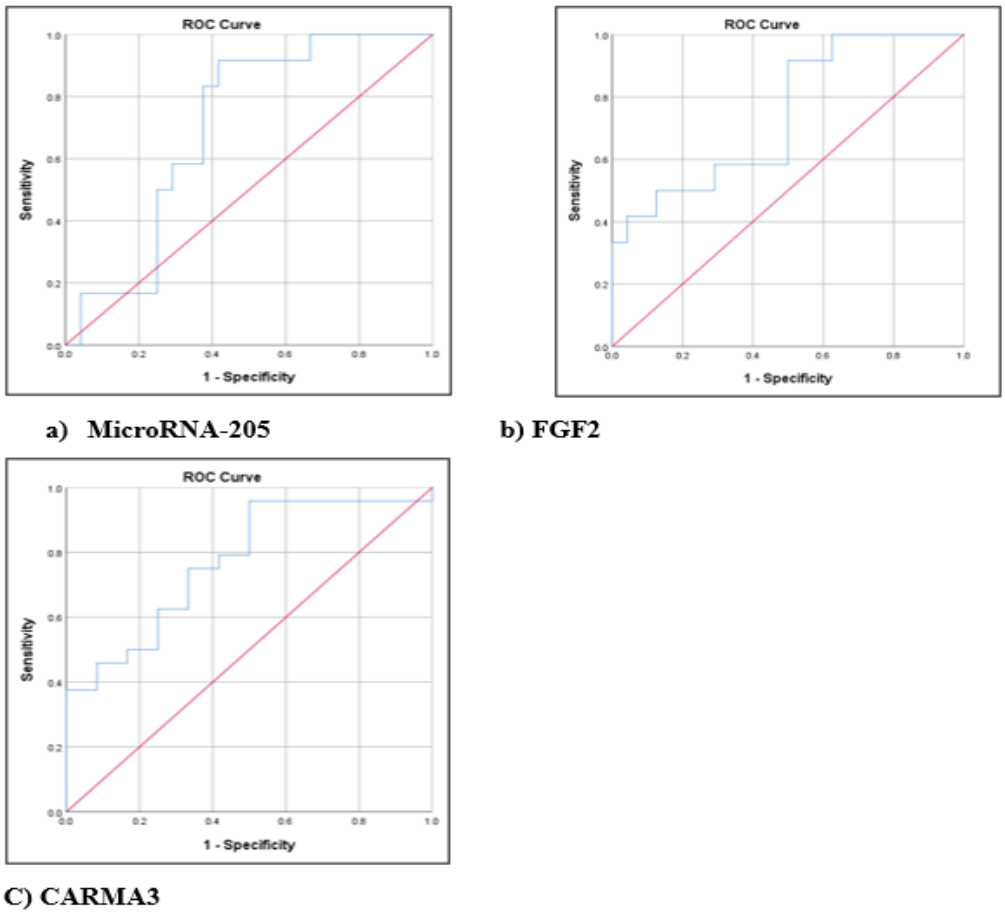


Figure 4. Receiver Operating Characteristic Curve of the (a) *MicroRNA-205*, (b) *FGF2* and (c) *CARMA3*.

Table 4. Using the $2^{-\Delta Ct}$ Method, the Expression Level of *CARMA3* was Determined in Control and Patient Groups

Groups	Means Ct of *CARMA3*	Means Ct of *GAPDH*	ΔCt (Means Ct of *CARMA3*)	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression
Patients	13.286	14.5875	-1.3008	2.4637	2.4637/2.0432	1.2
Control	13.229	14.26	-1.03	2.0432	2.0432/2.0432	1

Relative gene expression was determined using the Livak $2^{-\Delta Ct}$ comparative method, with U6 as the reference gene for miRNA (18).

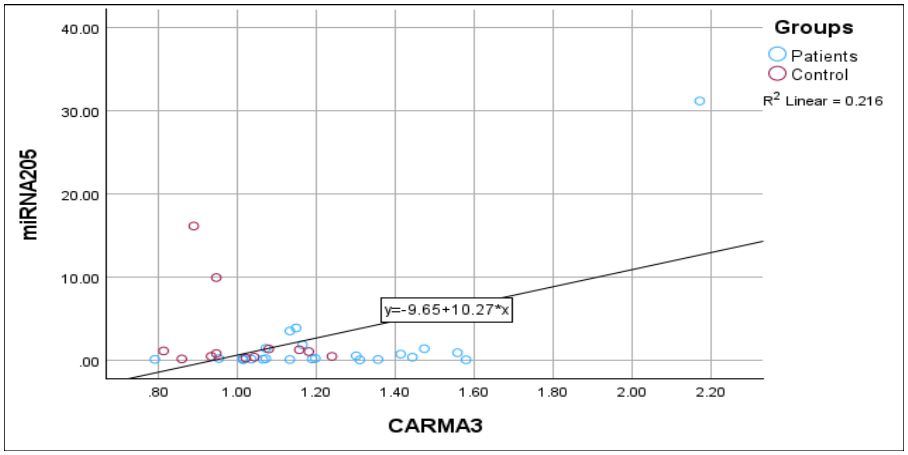


Figure 5. Positive Correlation between *CARMA3* and *miRNA205*.

Table 5. Demographic and Clinical Characteristics of Patients and Control

Characteristics	Patients (no.80)	Control (no.40)
Age (years)		
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		
Male (no)	50 (62.5%)	17 (42.5%)
Female (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	

Data were expressed as mean ± SD; Statistical analyses were performed by T-test. SD, Std. Deviation; NS, no significant difference.

0.004), while miR205 and *FGF2* and *FGF2* and *CARMA3* correlations were weak and non-significant ($p > 0.7$) (Table 7; Figure 5).

Discussion

Validation of reference gene in study groups

The idea behind using reference genes in molecular studies is that the cells or tissues being studied will continue to express them consistently [19]. One of the reference genes most commonly used in gene expression research is the *GAPDH* gene and U6 [20].

Real time polymerase chain reaction quantification of MicroRNA-205, FGF2 and CARMA3 expression

The downregulation of miR205 in CRC patients suggests its potential function as a tumor suppressor. This is supported by previous studies showing that miR205 can inhibit tumor growth and invasion by targeting carcinogenic pathways [8]. Reduced miR205 expression has also been associated with more aggressive tumors and poorer prognosis in CRC patients [21-23]. The observed downregulation of *FGF2* could be explained by tumor microenvironment variations or feedback mechanisms limiting expression [11]. Research indicates that *FGF2* expression changes are context-dependent and influenced by demographics, tumor stage, and grade [24]. The slight upregulation of *CARMA3* observed in this study suggests involvement in NF-κB pathway activation, which is crucial for inflammation and tumor growth [25]. This is consistent with findings linking NF-κB activation to increased cancer aggressiveness and apoptosis resistance [26, 27].

AGE and BMI

The absence of significant differences in age and BMI between the two groups reduces the likelihood of these factors acting as confounding variables in interpreting the gene expression results [28, 29].

Table 6. Receiver Operating Characteristic Curve Data of the Studied Gene

Parameters	AUC	P value	The best Cut off	Sensitivity %	Specificity%
MicroRNA-205	0.7	0.05	0.2275	91	58
FGF2	0.74	0.019	0.3556	91	50
CARMA3	0.77	0.009	0.9488	95	50

Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of each biomarker. The Area Under the Curve (AUC), optimal cutoff values, sensitivity, and specificity were calculated. A p-value < 0.05 was considered statistically significant.

Table 7. Pearson Correlation

		MicroRNA-205	*FGF2*	*CARMA3*
MicroRNA-205	r =	1	-0.057	.465**
	P =		0.741	0.004
FGF2	r =		1	-0.048
	P =			0.78
CARMA3	r =			1
	P =			

**, Correlation is significant at the 0.01 level (2-tailed); r, Pearson correlation; P, p-value; Pearson correlation analysis was performed to assess the relationship between the expression levels of the studied genes. A p-value < 0.05 was considered statistically significant.

Distribution of Gender

Although a higher percentage of males was observed in the patient group, the lack of statistical significance suggests that gender distribution is unlikely to have influenced the study findings [30].

WBCs

The non-significant differences in WBC counts suggest similar levels of immune cell presence and systemic inflammatory response between patients and controls, minimizing their potential confounding effect [31].

Cytokines IL-18 and IL-22

Comparable levels of IL-18 and IL-22 between patients and controls indicate that these cytokines may have limited diagnostic value in this population. These findings differ from some reports in other cohorts, highlighting the need for population-specific validation [32, 33].

The receiver operating characteristic curve

ROC analysis confirmed the diagnostic potential of the studied markers, with the balance between sensitivity and specificity being a key determinant of clinical performance [32, 33].

Despite miR205 limitations in terms of false positive rates, miR205 is a promising screening tool due to its high sensitivity but moderate specificity. Because healthy people may be mistakenly classified as positive, *miR-205*'s poorer specificity may limit its use in screening, even while it is helpful in identifying actual cancer cases [8]. These results are consistent with earlier research showing that miR205 functions as a tumor suppressor in certain cancers but has a dual function in colorectal cancer, contributing to both tumor suppression and advancement depending on the molecular background [7, 34].

FGF2 suggests a considerable diagnostic potential [11]. With high sensitivity in detecting positive cases, its ability to accurately differentiate between affected and healthy individuals is limited due to its relatively low specificity. Given its role in angiogenesis and cellular proliferation key processes in tumor development elevated *FGF2* levels are often linked to carcinogenesis [35]. Therefore, although *FGF2* may serve as a valuable marker for detecting colorectal cancer (CRC), its lower specificity highlights the need to combine it with other biomarkers to enhance diagnostic accuracy.

Among the three markers, *CARMA3* demonstrated the highest AUC, indicating a good diagnostic potential [14]. Despite its relatively low specificity which increases the risk of false positives, its exceptionally high sensitivity highlights its strong ability to accurately identify colorectal cancer patients [36]. Therefore, while *CARMA3* may benefit from being combined with other markers to improve overall diagnostic accuracy, its high sensitivity supports its potential as a reliable biomarker for colorectal cancer detection.

Sensitivity vs. Specificity

All three indicators demonstrated high sensitivity (between 91% and 95%), suggesting that they have a great deal of promise for application in colorectal cancer screening and early diagnosis. Their reduced specificity (between 50% and 58%), however, indicates a gap in their ability to differentiate CRC patients from healthy controls, perhaps leading to a greater number of false-positive results.

Comparative Analysis

CARMA3 had the highest AUC (0.77), indicating the best overall diagnostic performance among the three markers, followed by *FGF2* (0.74) and *miR-205* (0.7). This suggests that *CARMA3* may be the most promising single biomarker for CRC detection in the studied population [32, 33].

Clinical Implications

The results indicate that although *miR-205*, *FGF2*, and *CARMA3* may be useful as CRC diagnostic biomarkers, their modest specificity suggests that they may work better in a multi-marker panel than as stand-alone assays [37].

Pearson Correlation

Correlation between miR205 and CARMA3

Pearson correlation analysis revealed a moderate positive correlation between miR205 and *CARMA3*. This relationship suggests that the two genes may share a common signaling pathway or be co-regulated in colorectal cancer. Notably, this association may involve the activation of the NF-κB pathway, where miR205

has been implicated in modulating inflammatory and carcinogenic processes, and *CARMA3* is recognized as a key regulator [8, 28]. The moderate strength of this correlation indicates a potential synergistic effect in modulating immune responses and tumor progression during cancer development.

Correlation between miR205 and FGF2

Since miR205 and *FGF2* do not significantly correlate, it is likely that these two genes function independently in the tumor microenvironment of colorectal cancer. *FGF2* is mostly involved in angiogenesis and cell proliferation, whereas miR205 is more linked to cell differentiation and death [11, 12]. This suggests that, rather than directly interacting through gene regulation, miR205 and *FGF2* may have different roles in the pathophysiology of colorectal cancer.

Correlation between FGF2 and CARMA3

In colorectal cancer tissues, the lack of a significant association between *FGF2* and *CARMA3* suggests that their expression levels are independent of one another. This could be because they are implicated in different pathways; for example, *CARMA3* is involved in NF-κB-mediated inflammatory responses, whereas *FGF2* promotes angiogenesis [14, 27]. As a result, focusing on various pathways might call for separate therapeutic strategies.

Conclusion

Using blood samples from an Iraqi cohort, this study evaluated the diagnostic potential of *miR-205*, *FGF2*, and *CARMA3* in colorectal cancer (CRC) patients. The results showed significant downregulation of *miR-205*, decreased expression of *FGF2*, and a slight increase in *CARMA3*, highlighting their possible roles as biomarkers. ROC analysis identified *CARMA3* as having the best diagnostic performance; however, the limited specificity of all markers suggests they are best applied in a multi-marker panel. No significant differences were observed in demographic or clinical factors, confirming balanced groups and reliable expression results. Further studies with larger cohorts are recommended to validate these findings and explore their potential in early diagnosis and personalized treatment strategies for CRC.

artificial intelligence (AI)

Artificial intelligence (AI) approaches were used for designing primers targeting some genes, *FGF2*, *CARMA3*, and *MicroRNA-205* That are associated with colorectal cancer (CRC) in Iraqi patients. Advanced AI approaches were applied to enhance the precision of primer design and location detection of best places on genetic sequences of target genes.

Applied Tools

1- Primer3: The target genetic sequence was designed to develop an initial primers program (Primer3). Step one – Tm, appropriate length of the primer and mismatch were among the parameters that we took into account. The results were further enhanced using AI to ensure more specificity and minimize the chances of hairpins or primer-dimers generation [36].

2- To design primers more accurately, machines based on deep learning methods further including DeepPrimer: are also used. An AI-based examine then analyses the genetic sequence and chooses an optimum set of primer binding areas primarily based on its effectiveness in interacting with the goal DNA [38].

3- Benchling: A tool that lets users introduce AI components into an interactive workflow, was also used. It was implemented to evaluate the primer efficiency in genetic material, generate sequences, and allow bioinformatics analysis of data [39].

Design Methodology

Genetic sequences corresponding to the target genes (MicroRNA-205, *FGF2* and *CARMA3*) were inputted in AI algorithms as sketch for comparison with other genomic databases. Given this knowledge, the authors selected primers that were most similar to those in the human genome. AI also looked at prior experimental experience to increase the chance that primers would succeed in PCR testing [40].

Author Contribution Statement

Nada Nizar: Study conception, experimental design, and data collection. Noora Mustafa: Laboratory analysis and data validation. Asmaa Mhmood: Statistical analysis, interpretation of data, and manuscript preparation. -Mohanad Kareem: Critical revision and final approval of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

Scientific body approval / Student thesis

This study was conducted as part of an academic project under the supervision of Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq.

Availability of data

The data generated and analyzed during this study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author upon reasonable request.

Study registration

This study was not registered in a clinical trial or meta-analysis registry because it is a molecular observational study, not a clinical intervention.

Ethical approval

The study protocol was reviewed and approved by the Ethical Committee of Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq (Approval No.: 8962, Date: 2024/12/19). Written informed consent was obtained from all participants prior to blood sampling.

Conflict of interest

The authors declare that there are no conflicts of

interest related to this study.

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