RESEARCH ARTICLE

Evaluation of microRNA 92a Expression and Its Target Protein Bim in Colorectal Cancer

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Abstract

Background: Colorectal cancer is one of the most commonly diagnosed cancers and leading causes of malignancy-related deaths all over the world. MicroRNAs (miRNAs) can regulate more than 60% of human genes, including tumor-stimulating, and -suppressor genes. Therefore, they can promote cancer development and affect risk of malignancy. miR-92a overexpression in CRC enhances tumor proliferation, invasion, and metastasis through downregulating different pro-apoptosis proteins including Bim. This study aimed to assess the role of plasma miR-92a as non-invasive marker in CRC patients, outline correlation between plasma miR-92a and serum Bim, and determine their correlations with clinicopathological parameters in CRC and adenoma patients. **Methods:** A total of 54 newly diagnosed CRC patients, 15 colonic adenoma patients, and 15 age- and sex-matched control subjects were recruited in this study. Plasma miR-92a was assayed by TaqMan qRT-PCR and serum Bim was measured by ELISA. **Results:** Statistically significant overexpression of serum miR-92a was observed in CRC patients as compared to adenoma and control groups (p<0.001 each) and lower serum Bim in CRC patients as compared to adenoma and control groups (p=0.001, p <0.001 respectively). The ROC curve analysis showed excellent AUC for plasma miR-92a in discriminating CRC from control (AUC=0.994), and adenoma (AUC=0.993) groups with highest diagnostic performance in discriminating CRC from controls (at cutoff 1.43, sensitivity 98.1%, specificity 93.9%), and adenoma patients (at cutoff 1.78, sensitivity 92.6%, specificity 93.3%). The diagnostic performance in discriminating early from late CRC was good (at cutoff 15, AUC=0.641, sensitivity 61.2%, specificity 80%). A significant negative correlation was evident between plasma miR-92a and serum Bim both in adenoma and CRC groups (P<0.001 for both). Higher plasma miR-92a expression (r=0.275, p=0.044) and lower serum Bim (r=-0.299, p=0.028) were found to be correlated with late CRC stages. **Conclusion:** Circulating miR-92a and Bim could be promising, non-invasive diagnostic and prognostic biomarkers in CRC.

Keywords: miR-92a- Bim- CRC

Introduction

Colorectal cancer (CRC) is the third most prevalent cancer in males and second in females worldwide. Regarding mortality statistics, it represents the third leading cause of malignancy-related deaths in women and men, and the second prevalent cause of cancer deaths when men and women are combined together. Almost 50% of CRC patients present with metastasis at diagnosis time or develop it during disease progression (Cekaite et al., 2016).

In Egypt, the incidence rate of CRC is 5.1% in men and 4.7% in women. Egypt is having high global incidence of early CRC as 35% of Egyptian CRC patients were below age of 40 (Bader et al., 2020).

The currently used CRC diagnostic procedures and lab tests are either invasive and discomforting or show significantly low specificity and sensitivity (Ferrari et al., 2021), and that's why extensive research efforts are directed towards the finding of less invasive, more convenient, and cost-effective biomarkers with higher diagnostic accuracy to detect CRC patients at earlier resectable stages.

MicroRNAs (miRNAs) are small non-coding RNAs, 18–23 nucleotides long, which act as post-transcriptional regulators of gene expression (Qadir et al., 2019).

miR-92a gene is considered an integral part of miR-17-92 precursor cluster positioned at chromosome 13q31.3 (Mogilyansky and Rigoutsos, 2013). miR-92a and its currently determined gene targets have been involved in cell cycle control and cell signaling, and thus very necessary during all stages of mammalian development (Fang et al., 2017). Many studies have concluded that miR-92a is abnormally overexpressed in different malignancies including leukemia, colorectal, lung, and ovarian cancers (Mogilyansky and Rigoutsos, 2013).

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BCL2 interacting mediator of cell death (Bim) protein is one of the most important pro-apoptotic factors extensively expressed in normal tissues. The activated Bim could stimulate activation of Bax protein leading to apoptosis through mitochondrial pathway (Li et al., 2014). It has been reported as a target for miR-92a and its downregulation relives its tumor-suppressing activity and thought to have a vital role in promoted CRC angiogenesis and proliferation (Xuan et al., 2015; Sionov et al., 2015).

Up to our knowledge, this is the first study that correlates serum Bim levels with plasma miR-92a expression in CRC and colonic adenoma patients. This study aims to assess plasma miR-92a expression in CRC patients and outline correlation between plasma miR-92a expression and serum Bim protein in CRC patients and their clinicopathological parameters.

Materials and Methods

Patients

This study was done on 84 participants, including 54 newly diagnosed CRC Egyptian patients at different stages, 15 colonic adenoma Egyptian patients, and 15 age- and sex-matched controls. Patients were recruited from surgical oncology outpatient clinics at National Cancer Institute (NCI) hospital, Cairo University, from September 2016 till March 2018. The study was permitted by the Institutional Review Board (IRB) of the NCI, Cairo University. It was permitted according to Helsinki guidelines of studies performed on human beings and written consent was obtained from all study subjects before their enrollment. All patients were subjected to; full history taking and clinical examination, ordinary biochemical and markers investigations, imaging techniques in the form of: CT and MRI, and endoscopic studies with biopsy taking and histopathological examination. Samples were obtained from all patients prior to any therapeutic or surgical intervention. Participants' age showed as mean±SD of (50.7±15) years in CRC patients, (37.9±12.5) years in the adenoma patients, and (44.6±12.4) years in controls.

Sample Collection

Eight milliliters of venous blood were withdrawn under aseptic precautions, 4 millimeters were put into sterile vaccutainer tube containing K2 EDTA and centrifuged at 3400 rpm for 15 min at 4oc. Supernatant plasma was transferred to sterile, labeled Eppendorf tube. These plasma samples were stored at -20°C until RNA extraction followed by assay of miR-92a by qRT-PCR. The other 4 milliliters were allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 3400 rpm. the supernatant serum was collected and stored at -20oc until ELISA assay of all samples for Bim concentration and determination of serum CEA, CA 19.9 using Cobas e411 Autoanalyzer, Roche.

Analysis of expression level of miR-92a by qRT-PCR according to TaqMan protocol

Total RNA, including microRNAs, was extracted using the miRNeasy Mini Kit catalog#: 217184 (Qiagen, Germany), according to manufacturer's instructions.

Five µL of extracted total RNA were used as template for synthesis of cDNA performed using TaqMan® MicroRNA Reverse Transcription Kit (cat. no. 4366596) and TaqMan® MicroRNA Assay Primers manufactured by Thermo Scientific, USA. 15µL of reverse transcription reaction components were used as total volume. Each 15µl reaction consists of 7µl master mix, 3µl of miR-92a primer (5x), and 5µl of RNA sample.

The extracted RNA was quantified and its purity assessed using NanoDrop R (ND)-1000 spectrophotometer (NanoDrop Technology, WILM, USA). RNA concentration in samples was detected via absorbance at 260nm with mean concentration 13µg/ml (A260 = 2-21µg/ml), while purity was determined via absorbance ratios at 260/280 with accepted range at (1.8–2.1).

The miR-92a expression was measured by qRT-PCR using TaqMan® microRNA assay kit and TaqMan® Universal PCR Master Mix II kit catalog No. 4428175 (Applied Biosystems). We pipetted 10µl of TaqMan® Universal PCR Master Mix II, 1µl of gene-specific TaqMan® primer/probe mix, and 6.5µl of nuclease-free water. Then, 2.5µl of each cDNA were pipetted in the corresponding well to reach final volume of 20µl/well.

TaqMan qRT-PCR was performed in duplicate, and U6 snRNA was used as endogenous (reference) gene for normalizing miR-92a expression. The relative miR-92a expression was calculated using equation $2^{-\Delta\Delta CT}$ where $\Delta\Delta CT$= (CT miR-92a - CT U6) patient sample - (CT miR-92a - CT U6) control sample (Livak and Schmittgen, 2001).

Primer sequences: for miR-92a, forward-5'-ACACAGGTTGGGATCGGTTG-3', and reverse-5'-CAAACTCAACAGGCCGGGA-3'; and for U6, forward-5'-CTCGCTTCGGCAGCACA-3', and reverse-5'-AACGCTTCACGAATTTGCGT-3'.

Quantitative determination of Bim in serum samples by ELISA technique

Serum Bim was measured using sandwich ELISA Kit (catalog#: E-EL-H0582) supplied by eBioscience Corp. (ThermoFisher Scientific, USA) according to manufacturer's instructions.

Statistical Methods

In this study, data was tested using SPSS version 25 (SPSS Inc., Chicago, IL, USA). Numerical parametric data was expressed as mean ± SD, while non-parametric variables as median and range. Qualitative variables were expressed as frequency and percentage. Student T test was used to assess the difference between 2 study groups means. The difference of non-parametric variable between 2 study groups was assessed using Mann-Whitney U Test. To examine relation between qualitative variables, Chi-square or Fisher's exact test was used. Kruskal-Wallis test was used for assessing difference between more than two independent variables. To find correlation between two variables, Spearman's rho (r) was calculated. The

ROC curve was used to determine cut-off values and to analyze diagnostic utility of different markers. All p-values are tow-sided and values<0.05 was considered statistically significant.

Results

Comparison of studied markers' levels between all studied groups and according to clinicopathological parameter

This study was carried out on 54 CRC patients aged (50.7±15) years. Male patients were 28 (51.9%), while female patients were 26 (48.1%). Based on radiologic and colonoscopic and operative findings, the most common CRC site was the colon (27; 50%), followed by rectum (18; 33.3%), the mean tumor size was (7.8±2.5cm), the late CRC stages (III+IV) were the predominant (49; 90.7%), and the most prevalent histological grades were

grade II (44; 81.5%) followed by grade III (8; 14.8%) as shown in Table 1.

Plasma miR-92a represented as (median; range), showed significant upregulation in CRC patients (31.1; 1.2-9753.8) when compared with control (0.5; 0-2.3) and adenoma (0.6; 0.01-1.2) groups (p<0.001 for both). No significant difference was found in plasma miR-92a expression between adenoma and control groups (p=0.787) as shown in Table 1 and Figure 1. Despite the increase in miR-92a expression pattern from early (10.9; 2.4-402.9) to late (36.6; 1.2-9753.8) CRC stages, there was no significant difference found in its expression (p=0.303). Moreover, no significant difference in miR-92a expression found according to gender, Family History, tumor site, side of colonic tumor and pathological grade in CRC and adenoma groups as shown in Table 1.

Serum Bim represented as (median; range), showed
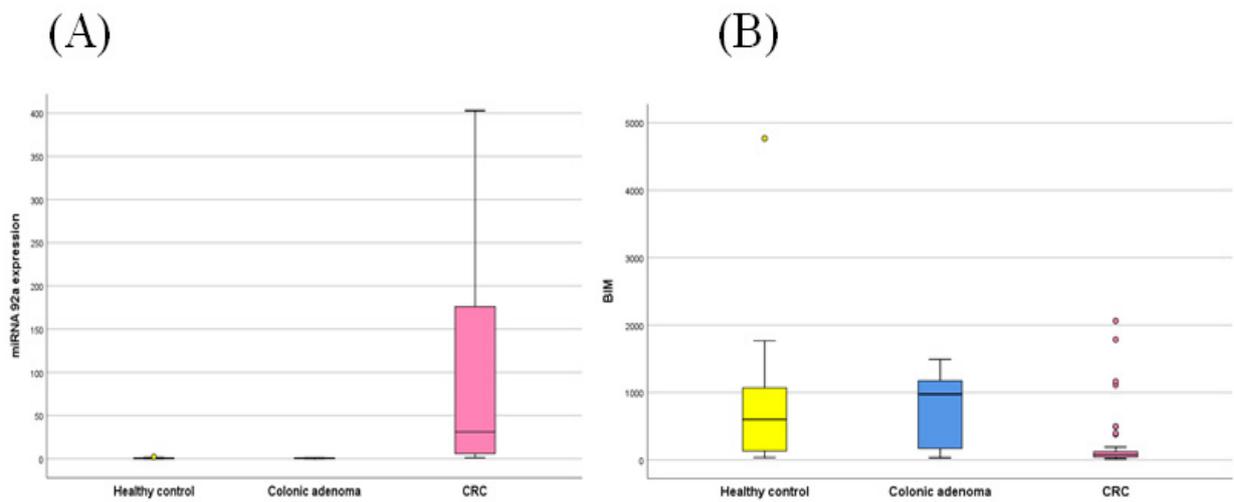
Figure 1. Box Plots for miR-92, Bim in CRC Patients Versus Adenoma Patients and Controls. miR-92a expression is significantly higher in CRC (A) compared to adenoma & control groups (p<0.001 each). Significantly lower Bim (B) in CRC compared to adenoma patients (p=0.001) and controls (p<0.001).
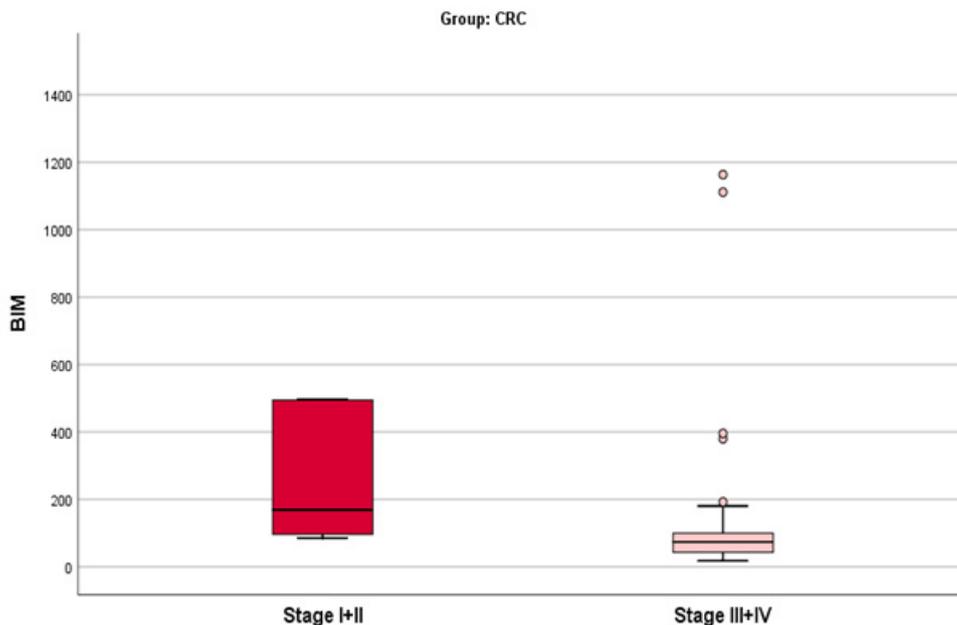
Figure 2. Serum Bim Concentration According to Stage in CRC Group.

Table 1. Comparison of Study Markers According to Clinicopathological Characteristics in CRC and Adenoma Patients

Parameter	Plasma miRNA-92a expression						Serum Bim (ng/ml)			
	In CRC group			In adenoma group			In CRC group		In adenoma group	
	N (%)	Median (range)	P value	N (%)	Median (range)	P value	Median (range)	P value	Median (range)	P value
Gender:	54 (100%)	31.1 (1.2-9753.8)	<0.001	15 (100%)	0.6 (0.01-1.2)	0.787	75.5 (17.9-2062)	<0.001	975.1 (32.8-1491)	0.604
Males	28 (51.9%)	14.7 (1.2-9753.8)	0.236	8 (53.3%)	0.76 (0.01-1.18)	0.418	58.4 (21.2-1785)	0.066	669.9 (32.8-1200)	0.355
Females	26 (48.1%)	61.4 (1.5-2560.7)		7 (46.7%)	0.26 (0.02-1.03)		90 (17.9-2062)		1077.6 (78.6-1491)	
Family History:										
Negative	39 (72.2%)	17.9 (1.2-9753.8)	0.961	NA	NA	NA	76.7 (17.9-2062)	0.854	NA	NA
Positive	15 (27.8%)	43.4 (1.5-783.3)		NA	NA		74.3 (19.6-1111.4)		NA	
Site of tumor:										
Colon	27 (50%)	15.5 (1.5-2912.5)	0.359	12 (80%)	0.36 (0.01-1.03)	0.096	85.2 (33-2062)	0.258	1026.4 (32.8-1491)	0.897
Rectal	18 (33.3%)	48.8 (2.9-9753.8)		2 (13.3%)	1.01 (0.84-1.18)		74.3 (17.9-494.6)		746.5 (480-1013)	
recto-sigmoid	9 (16.7%)	69.9 (1.2-2560.7)		1 (6.7%)	0.99		57.8 (19.6-1785)		364.6	
TNM staging:										
I+II	5 (9.3%)	10.9 (2.4-402.9)	0.303	NA	NA	NA	169.2 (85.2-498)	0.03	NA	NA
III+IV	49 (90.7%)	36.66 (1.2-9753.8)		NA	NA		74.1 (17.9-2062)		NA	
Modified Dukes Staging System										
B1	2 (3.7%)	12.9 (10.9-14.8)	0.347	NA	NA	NA	295.5 (96.3-494.6)	0.198	NA	NA
B2	15 (27.8%)	15.5 (2.4-402.9)		NA	NA		85.2 (34.2-2062)		NA	
C1	18 (33.3%)	84 (1.2-9753.8)		NA	NA		60.3 (17.9-379.8)		NA	
C2	19 (35.2%)	34.8 (1.5-2459.3)		NA	NA		76.7 (33-1785)		NA	
Histological grade										
Grade 1	1 (1.9%)	10.6	0.449	NA	NA	NA	100	0.74	NA	NA
Grade 2	44 (81.5%)	27.2 (1.5-9753.8)		NA	NA		67.7 (17.9-1163.2)		NA	
Grade 3	8 (14.8%)	39.1 (1.2-2912.5)		NA	NA		82.8 (33-2062)		NA	
Grade 4	1 (1.9%)	2459.3		NA	NA		87.4		NA	
Surgical margin:										
Free	34 (63%)	30.9 (1.2-9753.8)	0.506	NA	NA	NA	75.4 (17.9-2062)	0.506	NA	NA
Positive	14 (25.9%)	37.4 (2.5-2459.3)		NA	NA		80.8 (35.8-395.7)		NA	
No operation	6 (11.1%)	24.8 (2.1-816.6)		NA	NA		133.3 (44.2-1163.2)		NA	

NA, Not Applicable, P value <0.05 is significant
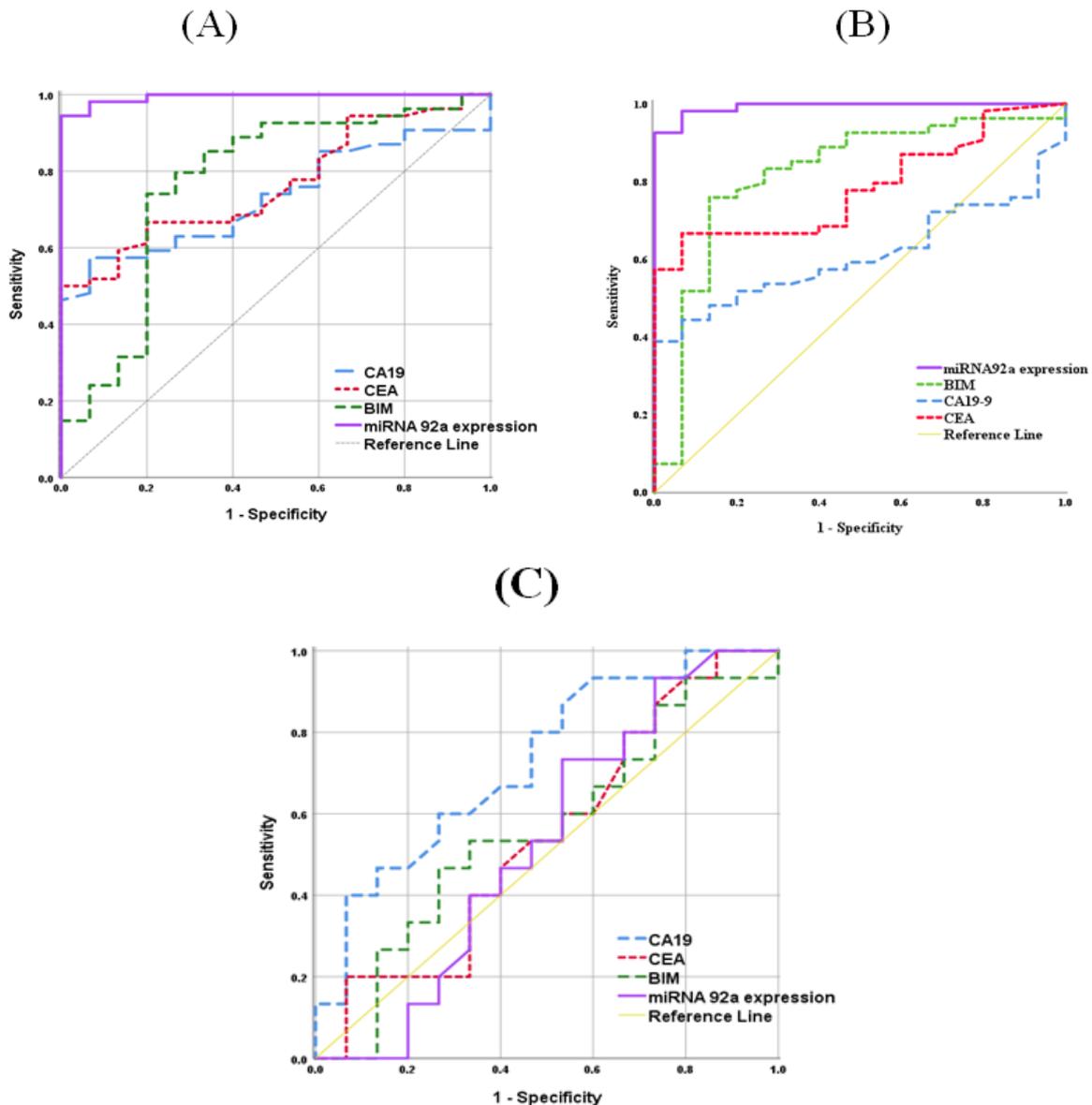
Figure 3. ROC Curves of Serum CA19-9, CEA, Bim Concentrations and Plasma miRNA92a Expression for Discrimination between Different Studied Groups. ROC curves of studied markers for discrimination between CRC patients and controls (A), CRC and adenoma patients (B), adenoma patients and controls (C).

significant downregulation in CRC patients (75.5; 17.9-2,062 ng/ml) when compared with adenoma (975.1; 32.8-1,491 ng/ml) and control (602; 36.6-4768.8ng/ml) groups (p=0.001, p<0.001 respectively) as shown in Figure 1 and Table 1. No significant difference was found in serum Bim levels between adenoma and control groups (p=0.604). Serum Bim showed significant lower levels in CRC advanced stages (74.1; 17.9-2,062 ng/ml) than in early stages (169.2; 85.2-498 ng/ml) (p=0.03). Otherwise, No significant differences were found in serum Bim according to clinicopathologic features of CRC and adenoma patients as shown in Table 1 and Figure 2.

Receiver Operating Characteristic (ROC) Curve analysis

The diagnostic utility of plasma miR-92a expression was evaluated by Receiver Operating Characteristic (ROC) curve analysis. The results shown in Table 2 and Figure 3, suggested that plasma miR-92a is the highest discriminator of CRC from adenoma patients (cutoff=1.78; AUC=0.993; Sn=92.6%; Sp=93.3%) and control subjects (cutoff=1.43; AUC=0.994; Sn=98.1%; Sp=93.9%).

Serum Bim showed the second highest diagnostic accuracy after miR-92a as it could differentiate CRC from colonic adenoma patients (cutoff<126.1 ng/ml; AUC=0.819; Sn=75.9%; sP=86.7%), and CRC from controls, (cutoff<110.1ng/ml; AUC=0.770; Sn=85.2%; Sp=66.7%).

While, serum Bim concentrations and plasma miRNA-92a expression showed failed AUCs (0.556, 0.529 respectively) and could not discriminate between adenoma and control groups.

Correlations of plasma miR-92a and serum Bim with clinicopathological features of CRC Group

Table 2. Diagnostic Performance Features of Studied Markers in All Studied Groups

Marker	Cut off	AUC	Sn%	Sp%	PPV%	NPV%	95%CI	Accuracy (%)	P value
1. Discrimination of CRC from the control group:									
CA19-9 (U/ml)	8.75	0.731	57.4	93.3	96.9	37.8	0.61-0.85	65.2	P^1 for CEA= 0.679
									P^1 for Bim= 0.795
									P^1 for miR-92a <0.001**
CEA (ng/ml)	4.9	0.765	66.7	80	92.3	40	0.65-0.88	69.6	P^2 for Bim= 0.919
Bim (ng/ml)	<110.1	0.77	85.2	66.7	90.2	55.6	0.62-0.92	75.4	P^2 for miR-92a=0.001**
miR-92a	1.43	0.994	98.1	93.9	98.1	93.2	0.98-1	97.1	P^3= 0.002**
2. Discrimination of CRC from the adenoma group:									
CA19-9 (U/ml)	15.9	0.612	44.4	93.3	96	31.8	0.48-0.74	55.1	P^1 for CEA= 0.043*
									P^1 for Bim= 0.041*
									P^1 for miR-92a <0.001**
CEA (ng/ml)	4.85	0.785	66.7	93.3	97.3	43.7	0.68-0.89	72.5	P^2 for Bim= 0.873
Bim (ng/ml)	<126.1	0.819	75.9	86.7	90.2	55.6	0.69-0.95	78.3	P^2 for miR-92a=0.001**
miR-92a	1.78	0.993	92.6	93.3	98	77.8	0.979-1	92.8	P^3= 0.002**
3. Discrimination of adenoma from the control group:									
CA19-9 (U/ml)	6.8	0.727	60	73.3	69.2	64.7	0.55-0.91	66.7	P^1 for CEA= 0.236
									P^1 for Bim= 0.176
									P^1 for miR-92a =0.196
CEA (ng/ml)	< 4.1	0.542	80	33.3	54.5	62.5	0.33-0.75	56.7	P^2 for Bim= 0.865
Bim (ng/ml)	943.1	0.556	53.3	66.7	61.5	58.8	0.34-0.77	60	P^2 for miR-92a=0.920
miR-92a	0.215	0.529	73.3	46.7	57.9	63.6	0.31-0.74	60	P^3= 0.944

AUC, area under ROC; ROC, receiver operating curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; P^1, comparison of AUCs versus CA19-9 AUC; P^2, comparison of AUCs versus CEA AUC; P^3, comparison of AUCs versus Bim AUC.

Statistical analysis using Spearman's correlation coefficient showed significant negative correlation between plasma miR-92a expression and serum Bim level both in adenoma and CRC groups (P<0.001 for both). Moreover, there was a significant positive correlation between plasma miR-92a expression and tumor staging (P=0.028). While, serum level of Bim showed a significant negative correlation with tumor staging (P=0.044).

Table 3. Correlations of miR-92a and Bim with Other Studied Parameters in CRC Patients

Parameter	Serum Bim		Plasma miR-92a	
	R	P value	r	p value
In CRC group:				
Age	-0.004	0.98	-0.127	0.359
Tumor Size	0.49	0.066	-0.355	0.163
Modified Dukes	-0.044	0.755	0.053	0.702
Histological Grade	0.079	0.572	0.121	0.383
TNM staging	-0.299	0.028	0.275	0.044
CA19-9	-0.019	0.889	-0.005	0.97
CEA	-0.008	0.954	0.129	0.351
Serum Bim	NA	NA	-0.95	<0.001
In Adenoma group:				
CA19-9	0.025	0.93	0.339	0.216
CEA	0.07	0.804	-0.222	0.426
Serum Bim	-	-	-0.904	<0.001

*P value <0.05 is significant

Otherwise, no significant correlations were found in serum Bim level and plasma miRNA 92a expression with age, tumor size, histological grading, and other serum markers in the studied groups as shown in Table 3.

Discussion

CRC is a heterogeneous carcinoma with markedly variable incidence and mortality rates globally (Ward et al., 2019). So, there is high need to find novel non-invasive biomarkers that can assist in identification of CRC in early stages, and help determine disease prognosis.

Our data showed that plasma miR-92a expression was significantly up-regulated in CRC patients when compared to both adenoma and control groups. This was in agreement with studies of Huang (2010); Liu (2013); Elshafei (2017); and Chen (2018), supporting that miR-92a is considered as an oncogene in CRC, and has the ability to promote tumor proliferation, migration and invasion.

This study results were also consistent with several studies performed on CRC tissues and stool samples. Wu (2012); and Lv (2016), reported that miR-92a expression was increased in tumor tissues, cell lines, and stools of CRC patients compared with that in corresponding adjacent normal tissues and stools of normal subjects.

Similarly, upregulation of miR-92a expression was reported in various malignancies including cervical cancer and osteosarcoma via targeting p21 and FBXW7 proteins, respectively (Su et al., 2017; Jiang et al., 2017), oral

squamous cell carcinoma by targeting FOXP1 expression (Guo et al., 2018), and NSCLC via targeting PTEN (Lu et al., 2017).

miR-92a regulates cell survival mechanisms by acting on different target genes. As an oncogene, it promotes tumor proliferation, metastases, and angiogenesis via inhibiting tumor apoptosis by suppressing the action of multiple proteins such as Bim (Penzkofer et al., 2014). Thus, its upregulation is strongly implicated in carcinogenesis and progression.

To extend miR-92a study in CRC pathogenesis, Bim was studied as one of its molecular targets. Up to our knowledge, no research studies evaluated the diagnostic performance of circulating Bim in serum or plasma of CRC patients. We found that serum Bim levels in CRC patients were significantly lower in comparison to adenoma and control groups. This was in agreement with studies done on CRC tissue samples by Greenhough et al., (2010); Wang et al., (2008); and Tsuchida et al., (2011).

Regarding association of miR-92a with clinicopathologic parameters of CRC patients, our results revealed significant association between its high plasma expressions with advanced tumor stage. This can support its role in invasion, and progression of CRC by repressing Bim protein (Penzkofer et al., 2014).

There was also significant association between serum Bim low levels and advanced tumor stage. On the other side, no significant correlation was found between either plasma miR-92a or serum Bim and other clinicopathologic features of CRC patients including age, tumor size, histological grading, or other serum biomarkers.

In agreement with our findings, Tsuchida et al., (2011) revealed that the higher miR-92a staining score using in-situ hybridization, and decreased Bim expression were directly correlated with increased incidence of lymph node metastases (P value <0.001 for both). Similarly, Elshafei et al., (2017) concluded that serum miR-92a expression was positively correlated (r =0.671, p =0.0001) only with advanced CRC stages. Other consistent studies showed significant positive correlation between miR-92a expression and advanced clinical and TNM staging with (p=0.008) (Thiery et al., 2009); (p=0.025) (Zhou et al., 2013); (p=0.008) (Zhang et al., 2014); (p=0.01) (Ke et al., 2015); (p= 0.002) (Song et al., 2016); and (p=0.001) (Li et al., 2016).

In several opponent studies to our results, Huang et al., (2010); Wang and Jin (2011); Schee (2012); Faltejskova (2013); and Yang (2014), found no significant correlation between miR-92a expression and TNM staging (P>0.05). Moreover, Sinicrope et al., (2008) concluded that there was no significant association of Bim expressions in CRC tissues with any clinicopathologic features.

Our study revealed significant negative correlation between plasma miR-92a expression and serum Bim in CRC and adenoma groups (P<0.001 for both) and correlation coefficient (r) = (-0.950, -0.904 respectively).

Several studies have implicated Bim as a target of miR-92a in CRC development (Tsuchida et al., 2011; Elshafei et al., 2017), glioma tumorigenesis (Niu et al., 2012), and lymphomagenesis (Ventura et al., 2008; Xiao et al., 2008). The up-regulation of miR-92a expression directly represses Bim protein which relives its tumor-suppressing action and thought to have important role in promoted angiogenesis in CRC (Elshafei et al., 2017).

Our study results suggested that plasma miR-92a expression could discriminate CRC patients from colonic adenoma patients and controls with excellent AUC, sensitivity, and specificity.

Serum Bim showed promising discriminator between CRC and adenoma patients, with good AUC, sensitivity, and specificity. While, its discriminating power is fair between CRC and controls.

Several agreeing studies including comprehensive meta-analyses showed that miR-92a yielded diagnostic sensitivity ranging (65.5%-89%), and diagnostic specificity ranging (64%-89%), and AUC ranging (0.772-0.940) indicating its moderate to high diagnostic power as convenient non-invasive detection method of CRC (Liu et al., 2013; Yang et al., 2014; Jiang et al., 2019; Peng et al., 2019).

The inconsistent miR-92a expression pattern in CRC and controversies in its correlation with other clinicopathological features could be attributed to sample types variations in different studies (Clancy et al., 2015), various detection methods of miR-92a (Fendler et al., 2016), and difference in subjects ethnicity (Elshafei et al., 2017)

In conclusion, plasma miR-92a expression was significantly upregulated in CRC patients when compared to adenoma patients and controls, which strongly suggests its promising role as potential non-invasive and reliable biomarker assisting diagnosis of CRC. Also, its plasma expression was negatively correlated with serum Bim, which was downregulated in CRC patients, and this may explain the miR-92a molecular effect on increased risk of CRC. miR-92a overexpression and low serum Bim, were associated with late tumor stages. So, they could help as prognostic markers in CRC.

Author Contribution Statement

The professors: Samia Y. Akel, Amal Fawzy and Reham A. Elshimy planed and designed the research; Ahmed Kamal Zaki participated in conception, interpretation of laboratory data and practical work. Hebatallah Gamal El Din and Ahmed Kamal Zaki collected clinical data and performed statistical analysis. All authors - contributing to the study design, participated in writing and editing final version of the manuscript, read and approved the final manuscript.

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Ethical Approval and Consent

The study was approved by the IRB of NCI, Cairo University. A written consent was obtained from all subjects before their enrollment.

Conflict of Interest

The authors declare no conflict of interest.

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