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ORIGINAL ARTICLE

Progressive Epigenetic changes at the level of the miRNAs in Colorectal Progression

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ABSTRACT

Serum carcinoembryonic antigen (CEA) and mRNAs (miR-20a, miR-145, miR-133b, miR-31) are known to be to tumor markers for colorectal cancer (CRC) development and progression. We aimed to determine the differential expression of CEA and miRNAs in CRC and correlate their expression levels with mRNAs of CRC-related genes (K-ras, P-53 and Let-7g). Twenty CRC tumour tissues from patients diagnosed with CRC stage II, III, IV and tissues from five healthy control subjects were analyzed. CEA and the relative miRNA expression of miR-21a, miR-145, miR-133b, miR31 and relative mRNA expression of K-ras, P-53, and Let-7g genes were determined with quantitative real-time PCR. The correlation between miRNAs and mRNAs in control and each tumour stage was determined. Our result showed high expression of CEA in tumour groups compared to control with no significant difference (P>0.05). There were significantly high (P<0.05) expression of miR-145, 133b and miR-31 in tumour groups compared to control and significantly high (P<0.05) mRNA expression of Let-7g and K-ras in control compared to tumour groups. Spear-rank correlation showed miR-31 and miR-145 are significantly (P<0.05) correlated in healthy subjects while there is positive correlation of miR-20a, miR-145, miR-133b and miR-31 in stage II and III, while in stage IV, miR-145 is highly correlated with miR-31 (P<0.05). There was no significant correlation (P > 0.05) betweenm iRNAs and relative mRNA expression of CRC genes. However, there was a significant correlation (P < 0.05) between CEA level and Let-7g in stage II. Overall, our findings support progressive changes at the level of CEA and miRNAs in colorectal progression that might provide unique biomarkers for patient risk. We suggest further studies on this.

Keywords: *Colorectal Cancer, CRC-related genes, miRNA,Serum carcinoembryonic antigen.*

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INTRODUCTION

Colorectal cancer (CRC) ranks the third most common cancer and the second most common cause of cancer death worldwide [1]. In Saudi Arabia, it is the first most common cancer among males and the third most common among females, according to the Saudi Cancer Registry (SCR) which estimated the incidence of CRC between January and December 2015, to be 12.2%, which accounts for 1465 newly diagnosed cases, with a predominance in males. The highest prevalence of CRC in Saudi Arabia has been reported in the capital, Riyadh [2] and it has been shown that the mortality rate of CRC in Saudi Arabia is high in comparison to other countries. For example, in 2018, the estimated worldwide mortality rate for both genders was 9.2%, while it was 15.2% in Saudi Arabia [3]. Also, a retrospective analysis of cancer registry data in 2015 reported that the 5-year survival rate of patients with CRC in Saudi Arabia was 44.6%, which is lower than the reported rate in the US (65.9%) [4]. However, CRC progression and metastasis can be prevented by detecting and removing precancerous polyps and also when diagnosed at an early stage [5]. Previous studies have reported several factors associated with an increased risk of CRC, including family history of CRC, old age, smoking, male gender, obesity, physical inactivity, and heavy

alcohol consumption (6). Inflammatory bowel disease has also been linked to a higher risk factor of CRC [7. Typical symptoms of CRC include changes in bowel habits, dark stool, rectal bleeding, abdominal pain, unintentional weight loss, and fatigue which usually appear at the late stage of the disease. Therefore, early screening is recommended for individuals at risk of developing CRC for effective therapeutic approach.

Tumor markers are biological or biochemical substances produced by tumor cells and then released into circulation at a detectable level. Antigens produced by the body in response to tumor growth or tumor markers produced by the tumor itself can both be beneficial markers for screening and staging [8].As an emerging therapeutic target and diagnostic biomarker, miRNAs play vital roles in tumor invasion, progression, and metastasis [9]. After their discovery, micro-RNAs (miRNAs) have been shown to have important implications in cancer biology. miRNAs function by binding to complementary sequences on the 3' untranslated regions, or the open reading frames of target genes to regulate gene expression at the post transcriptional level, leading to the degradation of target mRNAs or the inhibition of mRNA translation [10]. Increasing evidence have shown that dysregulated miRNAs' expression has a functional role in the progression and metastasis of colorectal cancer, acting either as tumor suppressors or oncogenes to regulate the expression of their specific mRNA targets. For example, Wu et al. showed that high levels of miR 18a in colorectal cancer attenuates the repair function of DNA and induce carcinogenesis by targeting ataxia telangiectasia mutated (ATM) gene to suppress ATM expression [11]. Also, it was revealed that overexpression of miR 19a in CRC cells promotes cell invasion and the epithelial mesenchymal transition (EMT) and is known to be associated with lymph node metastasis [12]. Therefore, studies on miRNAs were considered to be a new class of valuable biomarkers due to their high stability [13].

First described in 1965, carcinoembryonic antigen (CEA) is a colorectal cancer tumor marker [14] which the National Comprehensive Cancer Network, the American Society of Clinical Oncology, and the European Group on Tumour Markers recommend be measured preoperatively in patients with non-metastatic colorectal cancer [15]. It is well established that the elevated CEA levels are associated with metastases and recurrence, which prompt the suggestion by some investigators that it be included in the American Joint Committee on Cancer staging system [16]. Before widespread use of modern imaging, an elevated preoperative CEA level calls for additional investigation, such as liver scintigraphy, to detect metastases [17]. In the era of high-quality computed tomography (CT), the utility of measuring preoperative CEA is less obvious because an elevated preoperative CEA with a normal CT scan does not prevent surgery with curative intent. However, an elevated preoperative CEA level can be normalized after resection of the primary tumor [18].

A regional study from Saudi Arabia has shown the habits of late presentation of the disease among Saudi population compared to that in western countries [19]. Early Screening for CRC is an important determinant factor for prevention and early detection for effective treatment options. Moreover, early detection significantly lessen the financial burden that comes with treatment costs which is also correlated with the stage of the cancer (20). Also, it has been shown that early screening for CRC reduces both the incidence and mortality rates of the disease [21]. Considering the high incidence rate and the long duration between early and advanced stages of the disease, a CRC screening program may prove to be effective if implemented in Saudi Arabia [22].

However, certain barriers to implementing CRC screening have been reported, such as a lack of awareness, absence of symptoms, unavailability of doctors' recommendation, and fear of positive test results [19, 21]. Identifying these barriers in the screening of CRC is important for the successful implementation of the program. While extensive research has been conducted in other nations, there is limited evidence available from the region of Saudi Arabia [19, 21]. Therefore, the results of this study may assist policy makers and healthcare practitioners in Saudi Arabia to implement a national screening program for CRC which would support one of the Saudi (Vision, 2030) pillars, "a vibrant society with fulfilling lives" that focuses on providing preventive medicine services for citizens and encouraging them to benefit from primary healthcare [23].

MATERIAL AND METHODS

Study design, patient inclusion and exclusion criteria

This study is a case control study involving twenty colorectal cancer patients that were randomly sampled from a population of patients at the Oncology Center of King Abdullah Medical City at Makkah and Jeddah. Five apparently healthy control subjects without any known malignancy, chronic disease, or active inflammatory condition were also included for comparison. This study includes both male and female patients who were above 18 years of age and Saudi nationality. Individuals in this study were

presented with colorectal cancer with any of the tumour stages of 2, 3, and 4. Patients with stage-1 tumour were not included in the present study. Cancer patients with mental illness, drug, and alcohol abuse, patients who were under 18 years of age, and patients with underlying acute or chronic disease such as acute infection, kidney diseases, cardiovascular disorders, and rheumatological diseases were also excluded.

Assessment of patients

The complete medical history, physical examination, laboratory investigation, and clinicopathological features were obtained and recorded for all patients. Tumor staging was performed in conformity with the American Joint Committee on Cancer (AJCC) system, 7th edition Tumor, Lymph nodes, Metastasis (TNM) staging classification 17. All patients were examined by the same medical oncologist and general surgeon.

Blood sample analysis (whole blood for extracting microRNA and serum for measuring CEA)

Venous blood samples were collected into tubes which were coated inside with ethylenediaminetetraacetic acid (EDTA). Human peripheral blood cells were isolated immediately from leukocyte concentrates (buffy coats) by Ficoll-Paque density gradient centrifugation. Briefly, 3 mL Histopaque (Ficoll) was placed in a 15mL Falcon tube. With the leaking method, the blood (approximately 4 mL) was added to the scallop so that it would not mix with the Ficoll. It was centrifuged at 1600 rpm for 30 min. The buffy coat was transferred to a new Falcon tube, and 8mL of PBS was added. The supernatant was discarded after centrifugation for 12 min at 1600 rpm. It was then washed once with PBS and kept at −80°C until RNA isolation. Serum was collected using standard sampling tubes containing separation gel, Na_2-Heparin, K3-EDTA and sodium citrate plasma.

RNA extraction and cDNA Synthesis

RNA was extracted from the tissue samples using miRNeasy Kits (QIAGEN) in accordance with the manufacturer's instructions. The cDNA was synthesized using highly specific cDNA synthesis kit for qPCR (QIAGEN). The quantitative real-time polymerase chain reaction (qRT-PCR) was carriedout using QUANTIFAST® SYBR ® GREEN PCR KIT (QIAGEN).

Statistical analysis

Microsoft Excel and SPSS software version 22.0 were used for statistical analysis. Firstly, the difference in CEA in healthy control subjects, colorectal cancer stage II, stage III and stage IV were analyzed using Kruskal-Wallis test. Relative mRNA and miRNA expression in control and tumour group were analyzed by using Man-Whitney U test. Furthermore, the correlations of the relative mRNA and miRNA expressions in healthy control and colorectal cancer stages were analyzed by Spearman-rank correlation. Likewise, the correlation between patients who took drug and those that had surgical procedure was analyzed by using spearman-rank correlation. The relative expression levels of miRNAs and genes were normalized to that of RNU6B and GAPDH, respectively, as internal controls. The level of significance for statistical test was 0.05. Graphs were plotted with the aid of Graphpad prism.

RESULTS

Demographic and clinicopathological parameters in colorectal cancer patients

The demographic and clinicopathological parameters are presented in Table 1. This study included sixteen male patients and nine female systems. Among the twenty five patients, nineteen patients received chemotherapy.

CEA expression levels in healthy control subjects and CRC stage II, III and IV

Primer Sequence of miRNAs and target genes are presented in Table 2. The differences in CEA expression in healthy control, tumour stage II, III and IV were analyzed with Kruskal-Walis test. The results obtained are shown in Table 3. According to our results, there was no significant difference ($P > 0.05$) in the mean CEA values in control, stage II, III and IV.

CEA, Relative miRNa and mRNA expression in Healthy Control and Colorectal Cancer Groups

Results are presented in Table 3 and Figure 1 and2. The mean difference in expression of miRNA, mRNa and CEA in healthy control and tumour group were determined by Mann-Whitney u test. The mean rank value of miR-20a was high in tumour group (14.15) compared to the control (8.40), although with no significant statistical difference ($P > 0.05$). However, there was high significant difference in miR-145, miR-133b and miR-31 of tumour group compared to the control ($P < 0.05$). The mRNA expression of Let-7g and K-ras were higher in Control (15.40 and 16.20, respectively) compared to the tumour groups (12.40 and 12.20, respectively) with no significant difference ($P > 0.05$). Also, the mean rank of mRNA expression level in P-53 was higher in tumour group (13.50) compared to the control (11.00) with no significant difference ($P > 0.05$). There was a higher CEA expression in tumour group compared to the control with no statistical difference ($P > 0.05$).

Correlation of miRNA and mRNA expression Levels in Healthy Controls and Colorectal cancer group

Results are presented Table 4 The correlations of miRNA expression levels in the studied groups (healthy control, CRC stage II, III and IV) were compared using spearman-rank correlation and the results shown in Table 8. There was a significant perfect correlation (R2 = 1) in the expression level of miR-31 and miR-145 in control (P < 0.05). In CRC stage II, mIR-20a is significantly correlated with miR-145, miR-133b and miR-31 (P < 0.05). The level of miR-20a in stage III is significantly up-regulated with the expression level of miR-145, miR-133b and miR-31. In stage 4, miR-20a is significantly correlated (P < 0.05) with miR145, miR-133b and miR31 while miR 145 has a perfect positive correlation (R2 = 1, P = 0.05) with miR-31.

Correlation of miRNA expression Levels in Healthy Controls and Colorectal cancer

Results are presented in Table 5. The correlations between mRNAs were determined using Spearman-rank correlation and the results presented in Table 9. There was no correlation in relative mRNA expression in CRC stage II and III. However, there is a perfect positive correlation (R = 1.000, P < 0.05) between Let-7g and K-ras in control. Also, there is a significant negative relationship between K-ras and P-53 (R = -0.900, P < 0.037). In stage IV, there is a positive relationship between Let-7g and K-ras (R = 0.976, p < 0.05).

Correlation of CEA, miRNA and mRNA expression Levels in Healthy Controls and CRC stages II, III, IV

Results are presented in Table 6a, b, c, and d. The correlations of CEA, mRNAs and miRNAs in healthy control subjects and CRC stages II, III and IV are given in Table 10. In healthy subjects (Control), Let-7g is slightly positively correlated with miR-20a (R = 0.7), miR-145 (0.505), and miR-31 (0.505) with no statistically significance (P > 0.05). Likewise, K-ras is slightly correlated with miR-20a (R = 0.7), miR-145 (R=0.4), miR-31 (R = 0.4) with no statistical difference (P > 0.05). There is positive relationship between P-53 and miR-21a (R =0.6), negative correlation between P-53 and miR-133b (R= -0.3) with no statistically significant difference. In stage II, Let-7g is slightly positively correlated with miR-145, miR-133b and miR-31 (P> 0.05) whereas Let-7g is significantly negatively correlated with CEA (R = -0.900, R = 0.037). K-ras is slightly positively correlated with miR-20a, miR-145 and miR-31 with no statistically significant difference whereas it is significantly positively correlated with miR-133b (R = 0.900, P < 0.05). P-53 in stage II has a slight insignificant positive correlation with miR-21a, miR-145, miR-133b, miR-31 (P>0.05).In stage III, there is a slight positive relationship between Let-7g and CEA (R = 0.400, P > 0.05). K-ras also has a slight positive relationship with CEA (R=0.714, P > 0.05) and miR-145 is slightly correlated with CEA (R=0.643, P > 0.05).In stage IV, Let-7g is slightly correlated with miR-20a, miR-145, miR-133b and miR31 (P>0.05). K-ras has a slight negative relationship with miR-133b (R= -0.357, P>0.05).

Relationship between Chemotherapy and Surgical Resection

Using Spearman-rank correlation, there was a significant positive relationship between CRC patients who took drug and those that has surgical procedure (R= 0.554, P < 0.05) (Table 7).

Table 1. Demographic and clinicopathological parameters in colorectal cancer patients.

Variables	Clinicopathological parameters	Number of Sample (N = 25)
Age group	< 40	6
	40 -60	16
	>60	3
Gender	Male	16
	Female	9
Chemotherapy	Yes	19
	No	6
Surgical Resection	Yes	16
	No	9
BMI	< 20	3
	20-30	16
	> 30	6
DBM	Yes	9
	no	16
HPN	Yes	6
	No	19

Table 2. Primer Sequence of miRNAs and Genes		
miRNA/Gene	Forward sequence (5' → 3')	Reverse Sequence (3' → 5')
miR-20a	ACAGTAAAGTGCTTATAGTGCA	GTCCAGTTTTTTTTTTTTTTTCTACCT
miR-145	GAAGAGCTAGTAGGTTGGAT	GATTCCAGTTTTTTTTTTTTTTTAACT
miR-133b	TGAGTAAACAGCTTATAGTGCA	GTCCAGTTTTTTTTTTTTTTTCTACCT
miR-31	GATAGTAAAGTACTTATAGTGCA	CTGGACTTTTTTTTTTTTTTTCTAGCT
Let-7g	GCACTGAGTTAGTAGGTGGT	GATCCAGTTTTTTTTTTTTTTTAACTATGC
K-ras	AATCCGTGTGGGTCAGAGAG	GAAACAATAGCCACCCTCCTT
P-53	ATGGAGGAGCCGCAGTCAGAT	GCAGCGCCTCACAACCTCCGTC
RNU6B	AGTTATACAGCGCGGTAATG	GTCCAGTTTTTTTTTTTTTTTCGATC
GAPDH	GTGGTCTCCTCTGACTTCAAC	TCTCTCTCTTCCTCTTGTGCTCT

Table 3. CEA, Relative miRNa and mRNA expression in control and tumour groups.			
miRNA/gene	Control	Tumour	p-Value
miR-20a	8.40	14.15	0.129
miR-145	19.00	11.15*	0.042
miR-133b	6.23	21.29*	0.012
miR-31	20.7	10.74*	0.04
Let-7g	15.40	12.40	0.447
K-ras	16.20	12.20	0.303
P53	11.00	13.50	0.53
CEA	7.6	14.35	0.071
*= P < 0.05 vs. control			

Table 4. Spearman-rank Correlation of miRNA relative Expression in Control, CRC stage II, III & IV.			
miRNA	Correlations	Correlation Coefficient	P-Value
Control			
miR-31	miR-145	1.00	0.000
Stage 2			
miR-20a	miR145	0.90	0.037
	miR-31	0.90	0.037
miR-145	miR-133b	0.90	0.037
	miR-31	1.00	0.000
Stage 3			
miR-20a	miR-145	0.82	0.023
	miR-133b	1.00	0.00
	miR-31	0.9	0.037
miR-145	miR-133b	0.821	0.023
	miR-31	0.893	0.003
miR-133b	miR-31	0.929	0.003
Stage 4			
miR-20a	miR-145	0.881	0.04
	miR-133b	0.786	0.021
	miR-31	0.881	0.004
miR-145	miR-133b	0.929	0.001
	miR-31	1.000	0.000

Table 5. Correlation of mRNAs in Control, CRC stage II, III and IV			
	Control		
mRNA	Correlations	Correlation Coefficient	P-value
Let-7g	K-ras	1.000	0.000
K-ras	P-53	-0.900	0.037
	Stage 4		
Let-7g	K-ras	0.976	0.000

Table 6a. Correlation of mRNA, miRNA and CEA in Healthy Control Subjects			
mRNA/miRNA	Correlation	Correlation coefficient	P-value
Let-7g	miR-20a	0.700	0.188
	miR-145	0.400	0.505
	miR-31	0.4	0.505
K-ras	miR-20a	0.7	0.188
	miR-145	0.4	0.505
	miR-31	0.4	0.505
P-53	miR-20a	0.6	0.285
	miR-133b	-0.3	0.624
miR145	CEA	0.900	0.037*
miR-31	CEA	0.9	0.037*

Table 6b. Correlation of mRNA, miRNA and CEA Colorectal Cancer stage II			
mRNA/miRNA	Correlation	Correlation Coefficient	P-value
Let-7g	miR-145	0.300	0.624
	miR-133b	0.600	0.285
	miR-31	0.300	0.624
	CEA	-0.900	0.037*
K-ras	miR-20a	0.400	0.505
	miR-145	0.700	0.188
	miR-133b	0.900	0.037*
P-53	miR-20a	0.3	0.624
	miR-145	0.4	0.505
	miR-133b	0.3	0.624
	miR-31	0.4	0.505
	CEA	-0.700	1.880

Table 6c. mRNA, miRNA and CEA Correlation in Colorectal Cancer Stage III			
mRNA/mRNA	Correlation	Correlation Coefficient	P-value
Let-7g	CEA	-0.404	0.294
K-ras	CEA	0.714	0.071
miR-145	CEA	0.643	0.119

Table 6d. mRNA, miRNA and CEA Correlation in Colorectal Cancer Stage IV			
mRNA/miRNA	Correlation	Correlation Coefficient	P-value
Let-7g	miR-20a	0.452	0.260
	miR145	0.310	0.456
	miR-133b	0.571	0.139
	miR-31	0.310	0.456
K-ras	miR-133b	-0.357	0.385

Table 7. Spearman-rank correlation between chemotherapy and surgical procedure

Correlations[b]

			Chemotherapy	Surgical resection
Spearman's rho	chemotherapy	Correlation Coefficient	1.000	.554**
		Sig. (2-tailed)	.	.004
	surgical resection	Correlation Coefficient	.554**	1.000
		Sig. (2-tailed)	.004	.

**. Correlation is significant at the 0.01 level (2-tailed). b. List wise N = 25

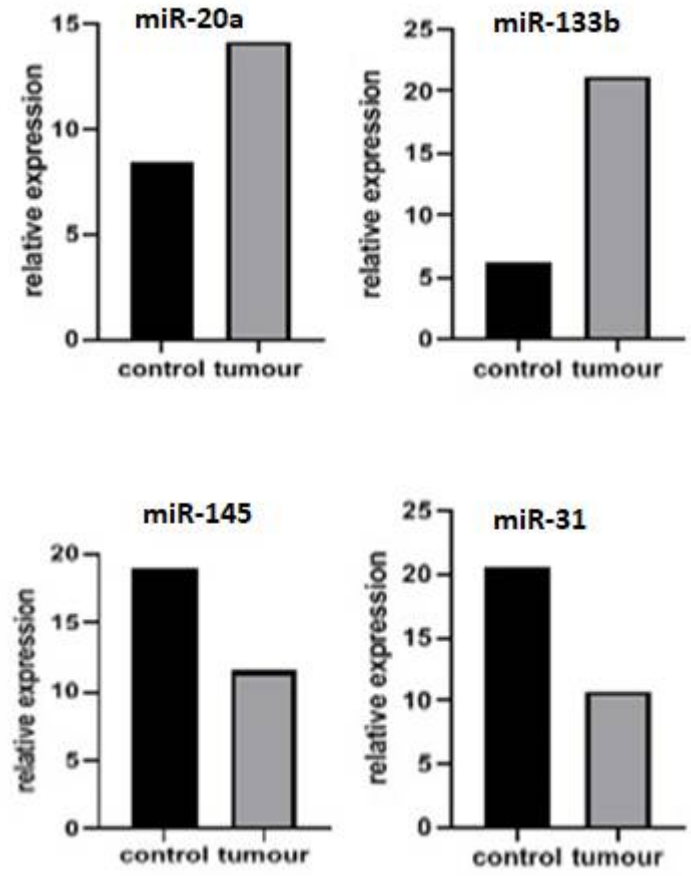

Figure 1: miRNA expression in control and tumour group.

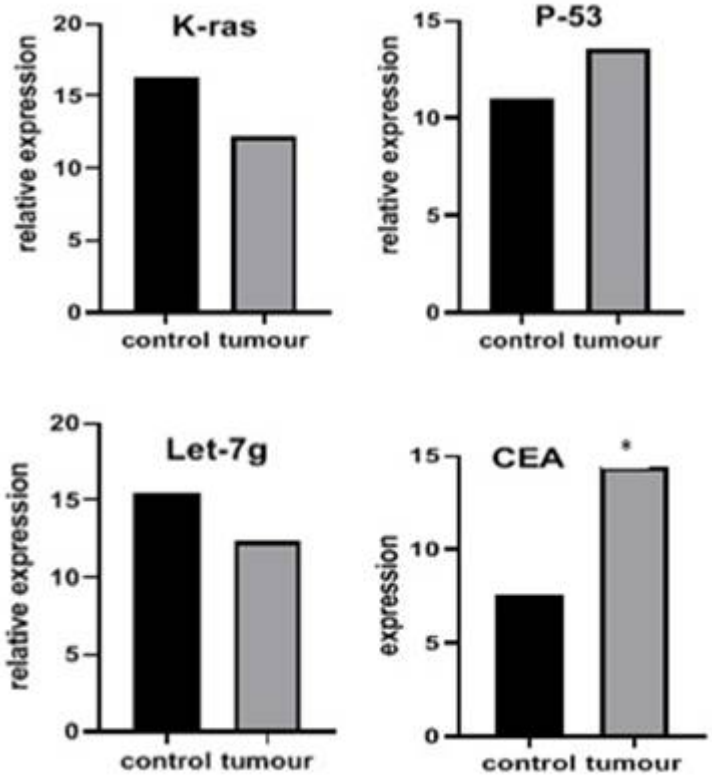

Figure 2: Relative mRNA and CEA expression in control and tumour group. ∗ = P < 0.05 vs control

DISCUSSION

It is known that miRNAs negatively regulate gene expression and promote abnormal expression in different kinds of malignancy. Many studies have shown the biological role of miRNAs in most signaling pathways of colorectal pathophysiology [24]. There are increasing body of research that cancer-related miRNAs are tumour suppressors and oncogenes in cancer pathogenesis [24]. Over the years, the roles of different miRNAs such as miR-20, miR-145, miR133b, miR31 has been well-studied in colorectal cancer pathogenesis in relation with the clinicopathological features of cancer patients [25].

The miRNA analysis and relationship with gene expression may be an important factor to determine the biology of colorectal cancer related miRNa and identification of their downstream targets, providing useful clues into deciphering the genesis and progression of colorectal cancer (26). Also, the potential of correlation might elaborate more on miRNA/mRNA interactive information arising from functional and computational studies. As a matter of fact, the experimental data from gene expression studies and miRNAs analysis possibly show miRNA-meidated regulatory mechanism for dysregulation of gene in cancer genesis and progression [27].

There is uncertainty about the clinical advantage of monitoring tumour marks in cancer patients (28). Serum CEA has not been recommended as a screening test but might been utilized preoperatively if it is proven effective in planning and staging treatment strategies. High serum CEA (> 5 ng/mL) indicates that patients have poor prognosis (29). However, there are not enough data to support the use of CEA as a determining factor for the use of adjuvant treatment (30). Studies on the Correlations between metastatic diseases and CEA in the past showed a relatively strong relationship with direct correlations. However, these relationships are totally gone, taken metastatic presentation into a partial correlation model. These can be explained by viewing CEA values as serving mainly as an approximation of the extent and the spread of disease as at the time measured. What seems to be a correlation in CEA and different long-term cancer outcomes turned out to be an indirect effect of the correlation between the preoperatibe CEA and the presence of metastatic disease. As in previous reports, our results concerning the relations of preoperative tumour marker levels with T and N stage suggest that a preoperative increase in the serum concentrations of these biomarkers might be a clue to lymphatic invasion. If the results of preoperative imaging studies are negative for lymphatic invasion, but elevated serum concentrations of tumour markers are present in a colorectal cancer patient, the physician might want to manage the patient as suspected of lymphatic invasion.

Different studies have demonstrated a decrease in CEA levels with metastatic cancer presentation as a covariate. Patients with advanced colorectal cancer have 2 predominant sites of metastasis which are the liver and the lung. Patients with metastases in these site tends to have more expression of CEA (31). Well-differentiated colon cancer cells with high surface expression of CEA may easily be picked up by the lungs or liver. In line with these studies, our result showed high expression of CEA in tumour grade II, III and IV compared to the healthy control, although with no statistically significant difference ($P > 0.05$), which means the lower the CEA level, the better the prognosis for colorectal cancer patients. We further determined the correlation between CEA level and mRNA/mRNA relative expression levels. We found a significant negative correlation between CEA level and Let-7g mRNA in CRC stage II ($P<0.05$) whereas CEA does not correlate with mRNa/miRNA in other CRC stages.

In this study, we determined the expression level of colorectal cancer-related mRNAs and some key genes, including LET-7g, K-ras, and P53. The relative expression of mRNA and miRNA in colorectal cancer samples was determined with quantitative real-time PCR technique. The different expressions of four miRNAs and mRNAs in 20 colorectal cancer patients was determined and correlated the corresponding miRNAs in 5 healthy subjects. It was found that relative mRNA expression in P53 and miRNA expression levels in miRNA20a were higher in tumour than in control, albeit no statistically significant difference. The relative expression level of miR-133b was significantly higher than that of control. In contrast, the relative mRNA expression in Let-7g and k-ras in control were higher than that of tumour group with no statistical difference. Accordingly, the miRNA expression level in miR-145 and miR-31 were significantly higher in control compared to the tumour group. This contradicts the findings of Moghadamnia et al. (32) where it was found that miR-31 was significantly more expressed in cancer samples compared to non-cancer samples. Also, Tsikitis et al. indicated that with colonic adenoma progression to a high-grade dysplasia and more advanced histology, miR-320a is overexpressed which is correlated with this study.

The mRNA expression level of let-7g were up-regulated in healthy control subjects in our study which is in line with the finding of Moghadamnia *et al*. that these miRNA and gene were downregulated in cancer samples compared with non-cancer group (32) . Also, Gao XH, et al. (33)demonstrated that k-ras expression levels were the highest in tumours and were correlated with the differentiation of tumour. Further analysis showed, in consistent with other results that the expression levels of k-ras are

significantly higher in advanced stages of tumour (III and IV) when compared with stage II tumour grade. This finding agrees with the fact that K-ras gene expression is the highest in GO and G1 stage of the cell cycle (34) . In contrast, our finding revealed that K-ras expression level is high in control compared to colorectal cancer patients additionally, we found that K-ras expression is highly correlated with the level of miR-133b ($P < 0.05$) in CRC stage II but not in advance stages of III and IV. Based on this, we can predict that miR-31 may have a more significant effect on K-ras mRNA expression levels in lower grade tumors than in advance stage tumors. In other words, higher K-ras mRNA expression level corresponds to a good prognosis for CRC patients. We further compared the correlation between patients who had history of surgical procedure and those were on chemotherapy where we found a significant correction ($P<0.04$) in these two groups of patients. These findings, taken together showed dysregulation of miRNAs in cancer samples compared with non-cancer samples.

CONCLUSION

We have observed differences in miRNAs and mRNAs expression levels during progression of colorectal cancer. Also, the level of carcinoembryonic antigen (CEA) was found to be increased in individuals with colorectal cancer as compared to the normal control subjects. However, miR-145, and miR-31 exhibits a behaviorconsistentwith tumor suppression with decreasing levels as the disease progresses while miR-20a and miR-133b are associated with disease progression. Similarly, the expression levels in Let-7g, K-ras appeared to be low in normal non-cancerous subjects in comparison to the tumour tissues. The role of miR-20a expression increases with premalignant disease progression. The role of miR-20a is still not well understood; our data and published studies suggest that miR-145 and miR-31 may serve as a defense mechanism against ‘‘invasiveness’’ and metastasis in CRC. We propose that further studies of miRNAs may give more clues on local defense mechanism against invasion. Overall, our findings support progressive epigenetic changes at the level of the miRNAs in colorectal progression that might provide unique biomarkers for patient risk stratification and may,through future mechanism studies, guide prevention efforts at specific events in early carcinogenesis in the colon and rectum.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Colorectal cancer statistics [Internet]. World Cancer Research Fund [Internet]. 2018. Available from: www.wcrf.org/dietandcancer/can cer-trends/colorectal-cancer-statistics.
2. SCR Annual reports [Internet]. 2018.
3. Estimated number of deaths in 2018, Worldwide, both sexes, both ages [Internet]. Global Cancer Observatory International Agency for research on cancer; 2018.
4. Zubaidi AM, AlSubaie NM, AlHumaid AA, Shaik SA, AlKhayal KA, AlObeed OA. (2015). Public awareness of colorectal cancer in Saudi Arabia: A survey of 1070 participants in Riyadh. Saudi J Gastroenterol. ;21(2):78-83.
5. Aljumah AA, Aljebreen AM. (2017). Policy of screening for colorectal cancer in Saudi Arabia: A prospective analysis. Saudi J Gastroenterol. ;23(3):161-8.
6. Cho YA, Lee J, Oh JH, Chang HJ, Sohn DK, Shin A, et al. (2019). Genetic Risk Score, Combined Lifestyle Factors and Risk of Colorectal Cancer. Cancer Res Treat. ;51(3):1033-40.
7. Herszenyi L, Barabas L, Miheller P, Tulassay Z. (2015). Colorectal cancer in patients with inflammatory bowel disease: the true impact of the risk. Dig Dis. ;33(1):52-7.
8. Pamies RJ, Crawford DR. (1996). Tumor markers. An update. Med Clin North Am. ;80(1):185-99.
9. Guo QR, Wang H, Yan YD, Liu Y, Su CY, Chen HB, et al. (2020). The Role of Exosomal microRNA in Cancer Drug Resistance. Front Oncol. 10:472.
10. Xi XP, Zhuang J, Teng MJ, Xia LJ, Yang MY, Liu QG, et al. (2016). MicroRNA-17 induces epithelial-mesenchymal transition consistent with the cancer stem cell phenotype by regulating CYP7B1 expression in colon cancer. Int J Mol Med. 38(2):499-506.
11. Wu CW, Dong YJ, Liang QY, He XQ, Ng SS, Chan FK, et al. (2013). MicroRNA-18a attenuates DNA damage repair through suppressing the expression of ataxia telangiectasia mutated in colorectal cancer. PLoS One. ;8(2):e57036.
12. Huang L, Wang X, Wen C, Yang X, Song M, Chen J, et al. (2015). Hsa-miR-19a is associated with lymph metastasis and mediates the TNF-alpha induced epithelial-to-mesenchymal transition in colorectal cancer. Sci Rep. 5:13350.
13. Mitchell PS, Kroh EM, Wyman SK, Parkin RK, Fritz BR, (2008). Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA. 105:10513–8.

14. Gold P, Freedman SO. (1965). Demonstration of Tumor-Specific Antigens in Human Colonic Carcinomata by Immunological Tolerance and Absorption Techniques. J Exp Med. 121:439-62.
15. Clinical Practice Guidelines in Oncology: Colon Cancer, (2016).
16. Kim CW, Yoon YS, Park IJ, Lim SB, Yu CS, Kim JC. (2013). Elevation of preoperative s-CEA concentration in stage IIA colorectal cancer can also be a high risk factor for stage II patients. Ann Surg Oncol. ;20(9):2914-20.
17. Szymendera JJ, Wilczynska JE, Nowacki MP, Kaminska JA, Szawowski AW. (1982). Serial CEA assays and liver scintigraphy for the detection of hepatic metastases from colorectal carcinoma. Dis Colon Rectum.2;25(3):191-7.
18. Tsai HL, Huang CW, Chen CW, Yeh YS, Ma CJ, Wang JY. (2016). Survival in Resected Stage II Colorectal Cancer Is Dependent on Tumor Depth, Vascular Invasion, Postoperative CEA Level, and The Number of Examined Lymph Nodes. World J Surg. ;40(4):1002-9.
19. Alamri D S, Alharbi D M, Alqahtani D S, Eltwansy D, D. SM. (2017). Public awareness of colorectal cancer and perceived barriers to its screening among attendees of primary health care centers in the eastern province of Saudi Arabia. Int J Sci Res.;6(10):590-97.
20. Kriza C, Emmert M, Wahlster P, Niederlander C, Kolominsky-Rabas P. (2013). Cost of illness in colorectal cancer: an international review. Pharmacoeconomics. ;31(7):577-88.
21. Almadi MA, Mosli MH, Bohlega MS, Al Essa MA, AlDohan MS, Alabdallatif TA, et al. (2015). Effect of public knowledge, attitudes, and behavior on willingness to undergo colorectal cancer screening using the health belief model. Saudi J Gastroenterol. ;21(2):71-7.
22. Galal YS, Amin TT, Alarfaj AK, Almulhim AA, Aljughaiman AA, Almulla AK, et al.(2016). Colon Cancer among Older Saudis: Awareness of Risk Factors and Early Signs, and Perceived Barriers to Screening. Asian Pac J Cancer Prev. ;17(4):1837-46.
23. Saudi Vision 2030. [Internet]. Vision2030.gov.sa. (2020). [cited 22 February 2020] available from: https://vision2030.gov.sa/download/file/fid/417. .
24. Catalanotto C, Cogoni C, Zardo G. (2016). MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. Int J Mol Sci. ;17(10).
25. Yu Y, Nangia-Makker P, Farhana L, S GR, Levi E, Majumdar AP. (2015). miR-21 and miR-145 cooperation in regulation of colon cancer stem cells. Mol Cancer. 14:98.
26. Wu XM, Shao XQ, Meng XX, Zhang XN, Zhu L, Liu SX, et al.(2011). Genome-wide analysis of microRNA and mRNA expression signatures in hydroxycamptothecin-resistant gastric cancer cells. Acta Pharmacol Sin. 32(2):259-69.
27. Zhou K, Liu M, Cao Y. (2017). New insight into microRNA functions in cancer: oncogene–microRNA–tumor suppressor gene network. Frontiers in molecular biosciences. ;4.90
28. Morita S, Nomura T, Fukushima Y, Morimoto T, Hiraoka N, Shibata N. (2004). Does serum CA19-9 play a practical role in the management of patients with colorectal cancer? Dis Colon Rectum. ;47(2):227-32.
29. Harrison LE, Guillem JG, Paty P, Cohen AM. (1997). Preoperative carcinoembryonic antigen predicts outcomes in node-negative colon cancer patients: a multivariate analysis of 572 patients. J Am Coll Surg. 185(1):55-9.
30. Bast RC, Jr., Ravdin P, Hayes DF, Bates S, Fritsche H, Jr., Jessup JM, et al.(2001). 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol. 19(6):1865-78.
31. values Jessup JM, Giavazzi R, Campbell D, Cleary K, Morikawa K, Fidler I. (1998). Growth potential of human colorectal carcinomas in nude mice: association with the preoperative serum concentration of carcinoembryonic antigen in patients Cancer Research. 48:1689-92.
32. Moghadamnia F, Ghoraeian P, Minaeian S, Talebi A, Farsi F, Akbari A. (2020). MicroRNA Expression and Correlation with mRNA Levels of Colorectal Cancer-Related Genes. J Gastrointest Cancer. 51(1):271-9.
33. Gao XH. (2017). Differences of protein expression profiles, KRAS and BRAF mutation, and prognosis in right-sided colon, left-sided colon and rectal cancer. Sci Rep. 7(1).11-14
34. Boutin AT, Liao WT, Wang M, Hwang SS, Karpinets TV, Cheung H, et al. (2017). Oncogenic Kras drives invasion and maintains metastases in colorectal cancer. Genes Dev. 31(4):370-82.