Egypt. J. Chem. **Vol. 68**, No. 5 pp. 455 - 464 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

The Dual Effect of Long Non-Coding RNA Hoxa Transcript at the Distal Tip and MiR-216a on Colorectal Cancer

Doaa A. Roushdy [1], Olfat G. Shaker [2], Wafaa Gh. Shousha [1], Manar S . Fouda[1], Khaled R. Diab [3], Sara M. Abdo[1*]

[1]*Biochemistry Division, Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt*

[2]*Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Giza, Egypt*

[3]*Department of General Surgery, Faculty of Medicine, Fayoum University, Fayoum, Egypt*

Abstract

Background: Hoxa transcript at the distal tip (HOTTIP) plays an oncogenic role in multiple cancer types, including colorectal cancer (CRC). Single nucleotide polymorphisms (SNPs) may impact the expression and function of HOTTIP; nevertheless, limited studies have explored the correlation between HOTTIP SNP and CRC. This study aimed to assess the diagnostic performances of non-coding RNAs HOTTIP and miR-216a expressions among CRC patients, in addition to investigating the genetic links between CRC susceptibility and HOTTIP SNP rs3807598. Methods: 50 CRC cases and 50 controls were encompassed in the study. HOTTIP and miR-216a were quantified using qRT-PCR. Genotyping for HOTTIP rs3807598 was carried out utilizing the TaqMan allelic discrimination test by Real-time PCR. Results: Compared to healthy individuals, the CRC patients exhibited significantly down-regulation in miR-216a expression and up-regulation in HOTTIP expression levels. The ROC curve analysis indicated reliable diagnostic performances of both serum miR-216a and HOTTIP among CRC patients (AUC= 0.87 and 0.94 respectively, $p<0.0001$). Furthermore, lncRNA HOTTIP SNP rs3807598 (C:G) was shown to be substantially linked to increased risk of CRC Significantly. Also the GG genotype showed significantly elevated expression profile of miR-216a and HOTTIP among HOTTIP genotypes. Conclusion: Based on these results, miR- 216a and HOTTIP could serve as biomarkers for CRC early diagnosis.

Keywords: Colorectal cancer, CRC, miR-216a, HOTTIP, polymorphisms, SNPs, rs3807598, biomarker

1. Introduction

One of the most common malignant growths and the major cause of cancer-associated mortality worldwide is colorectal cancer (CRC) [1]. Genetic and environmental risk factors both affect the development of colorectal cancer [2]. The incidence of CRC rose as a result of alterations in lifestyle, environmental changes, and aging populations [3]. The survival rate for individuals with advanced colorectal cancer is still quite low, despite recent advancements in surgical and multimodal cancer therapy [1]. Therefore, it is necessary to find novel biomarkers for the diagnosis and prognosis of CRC.

A diverse collection of transcripts known as non- coding RNAs (ncRNAs) are by definition not translated into proteins. According to human genome sequence data, over 90% of DNA sequences are actively transcribed, but only 2% of them produce protein, hence the bulk of transcripts are ncRNAs [4]. Since their discovery, non-coding RNAs (ncRNAs) have been shown to be significant regulators of numerous biological processes in a variety of cell types and tissues; their dysregulation has been linked to various diseases. Among these are circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), and microRNAs (miRNAs) [5]. They have been found to be tumor suppressors and oncogenic drivers in a variety of cancers.

More than 60% of human coding genes may negatively regulated by microRNA whereas, long noncoding RNAs regulate gene expression on several levels by interacting with functional proteins, chromatin, and RNAs such mRNAs and microRNAs [6]. LncRNAs are a type of ncRNAs molecules that has a length of more than 200 nucleotides. They are transcribed in the genome and act as regulators in a variety of biological processes. Abnormal expression of LncRNAs participates in the development of a variety of malignancies, including CRC [7]. HOXA transcript at the distal trip (HOTTIP) is a novel lncRNA that is transcribed from the 5′ tip of the HOXA locus and promotes the activation of various HOXA genes, therefore accelerating oncogenesis [8]. HOTTIP is a potential biomarker and treatment option in human malignancies, as well as an oncogenic lncRNA in almost all types of cancers [9]. For instance, renal cell carcinoma, up regulation of HOTTIP gene

*Corresponding author e-mail: sara.Mohamed@science.helwan.edu.eg.; (Sara M. Abdo).
Received date 30 May 2024; Revised date 27 June 2024; Accepted date 01 July 2024
DOI: 10.21608/ejchem.2024.293873.9788

inhibits the expression of tumor suppressor gene (LATS2). Down regulation of LATS2 gene leads to enhancement of cell growth [10]. The interaction of HOTTIP with the WDR5/MLL complexes has a role in the pathogenesis of human esophageal squamous cell carcinoma, pancreatic cancer, and gastric cancer [9].

Some lncRNA polymorphisms have been found to be helpful in predicting cancer risk. The role of long noncoding RNAs (lncRNAs) can be changed by single nucleotide polymorphisms (SNPs), either promoting or inhibiting the development of illness. By interfering with transcription factor binding, SNPs can directly affect the expression of lncRNAs or directly affect the expression of regulatory factors. Additionally, SNPs have the potential to change how lncRNAs associate with other RNAs or proteins. [8]. MicroRNAs (miRNAs) are a class of small noncoding RNA molecules that regulate gene expression by blocking or stimulating mRNA translation and destruction, and then participate in several essential physiological processes including Cell development, differentiation, invasion and metastasis [11]. Mutations in the biosynthesis of these miRNAs have been linked to the development of cancer [12]. MiRNAs have been found to influence EMT-mediated metastasis**.** The change of cells from epithelial to mesenchymal phenotype (EMT) accelerates cancerous cells' migratory and invasive characteristics, resulting in tumor metastasis. Over-expression of miR-216a-5p can inhibit cell proliferation, invasion, as well as the EMT pathway in CRC cells by silencing YBX1 expression [13].
The main objectives of the study were to evaluate the significance of HOTTIP and miR-216a expression levels, along with HOTTIP rs3807598 genotyping, as diagnostic and prognostic markers in Egyptian patients with colorectal cancer.

2. Methods

One hundred Egyptian individuals were chosen for this study from department of general surgery Fayoum University. Every participant underwent a colonoscopy to screen for colorectal cancer and to check for symptoms of the lower gastrointestinal tract, such as chronic constipation, diarrhea,and bleeding that occurs in rectum. The healthy control group includes fifty individuals with no CRC history, negative colonoscopy results IBD, polyps, or malignancy. The CRC group includes fifty patients diagnosed with CRC based on pathology results and a positive result of colonoscopy. Every individual provided their complete case history, and standard laboratory tests and clinical investigations were also conducted. Every patient completed an informed consent form, and the ethics committee at Fayoum University faculty of medicine approved it. Exclusion criteria: patients with inflammatory bowel disease (IBD), patients who had previously exposed to radiotherapy or chemotherapy for CRC, patients diagnosed with cancer at another site during the study. A miRNeasy mini kit and procedure for purification of serum total RNA, including non- coding RNAs (Qiagen, valecia, CA, USA) were used to extract RNA from serum. Total RNA is purified using a silica membrane after samples are lysed using phenol/ guanidine in the miRNeasy Mini Kit. QIAzol Lysis Reagent is intended to promote sample lysis,inhibit RNases, and to eliminate most of the cellular DNA and proteins from samples by using organic extraction. The homogenate is divided into three layers by the addition of chloroform: upper aqueous layer in which the RNA concentrates, an intermediate layer contains DNA, and third lower organic layer of denatured proteins. The upper, aqueous layer is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules. The sample is then applied to the RNeasy Mini spin column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. RNA is then eluted in RNase-free water. The extraction of DNA from mononuclear cell layer using QIAamp kit from Qiagen (USA, catalogue number 51306) according to manufacturer's instructions. Genotyping was performed using real-time polymerase chain reaction with TaqMan allelic discrimination assay (Applied Biosystems, USA). A predesigned primer/probe set (HOTTIP) for the genotype was used (Applied Biosystems, USA). Probe was synthesized with reporter dye FAM or VIC covalently linked at the $5'$ and a quencher dye MGB linked to the $3'$ end of the probe (Applied Biosystems, USA).

Statistical Analysis

The statistical package for social science (SPSS v23) was used to analyze the data. For qualitative data, descriptive analysis was carried out using percentages and numbers. The mean and stranded deviation (SD) of quantitative parametric data was displayed. The One-way ANOVA test was utilized to compare measures of more than two indep endent groups, and the Benferroni Post-Hoc was applied to test significance at p-value ≤ 0.05. The independent student t-test was employed to compare measure of two independent groups. The Mann- Whitney test was employed to determine the significance of the difference between more than two independent groups in quantitative non parametric data, while the Kruskalwallis test was utilizes to compare more than two separate groups. For data that is qualitative, to determine the relationship between various groups, use bivariate Pearson correlation test with a two- tailed significance test. Sensitivity and specificity test were created for testing a new test with ROC Curve (Receiver Operating Character). P- value ≤ 0.05 was measured as a cutoff value for significance.

3. Results

3.1. Demographic and clino-pathological features within the studied groups

Demographic, clino-pathological, laboratory as well as colonoscopic features in CRC and control groups were illustrated in Table 1. Data clarified that Hg level decreased significantly within CRC group compared to the control group (p=0.05) while the other laboratory parameters such as, ALT, AST, BIL, and Albumin decreased insignificantly. Clinical and pathological parameters showed that abdominal pain and constipation were the most common symptoms in CRC patients than other symptoms demonstrated in Table 1. The site of tumor in (Rectum+Sigmoid) colon represent (29; 50.9%), (Transverse+Flexures) represent (16; 28.1%), and (Ceacum+Ascending) had (12; 21.0%). Other pathological features from colonoscopy picture illustrated that mass in CRC found in (37; 64.9%), ulcer (10; 17.5%) and Hyperemia (2; 3.5%). The CT analysis showed fifteen cases of patients (26.3%) had mass lesion, thirteen cases (22.8%) had wall thickening, and seventeen cases (29.8%) suffered from regional lymph node, three cases found in (5.3%) with liver metastasis.

Table 1: Demographic and clino-pathological features of the studied groups

Variables	Healthy controls	CRC	P-value
Age (years)	50.24±0.783	51.89±1.581	0.371
Sex	50(29M/21F)	50(34M/16F)	0.268
Symptoms of presentation			
Abdominal Pain		39(68.4%)	
Constipation		39(68.4%)	
Loss of weight		27(47.4%)	
Bleeding per rectum		15(26.3%)	
Microcytic Anemia		11(19.3%)	
Hg (g/dL)	12.50±1.1	**10.98[a] ± 2.94**	**0.05**
Platelets	177±100.0	185.551±131.827	0.105
ALT (0-42 IU/L)	22.2±9.07	21.925±10.07	0.779
AST (0-42 IU/L)	25.67±8.1	24.125±10.69	0.872
BIL (mg/dL)	0.88±0.15	0.74±0.34	0.494
Albumin (3.5-5.5 g/dL)	4.73±0.4	4.43±0.94	0.6
Type of Tumor			
Adenocarcinoma		47(82.5%)	
Mucoid		10(17.5%)	
Site of tumor			
Ceacum+Ascending		12(21.0%)	
Transverse+Flexures		16(28.1%)	
Rectum+Sigmoid		29(50.9%)	
Colonoscopy Picture			
Colonoscopy Mass		37(64.9%)	
Colonoscopy Ulcer		10(17.5%)	
Colonoscopy Hyperemia		2(3.5%)	
CT Picture			
Mass Lesion		15(26.3%)	
Wall Thickening		13(22.8%)	
Regional LNs		17(29.8%)	
Liver Metastasis		3(5.3%)	

Values are expressed as mean ± SD or number (percentage). CRC: colorectal cancer; Hg, hemoglobin; ALP, alkaline phosphatase; ALT, alanine transaminase; BIL, bilirubin. Data are considered to be statistically significant at ($p \leq 0.05$).

3.2. Serum biomarkers HOTTIP and miR-216a in CRC

The CRC samples exhibited significantly lower miR-216a expression relative to normal control samples (0.39±0.069 versus 0.99±0.01, p=0.0001), on the other hand the expression level of HOTTIP highly increased significantly within CRC than the control samples (5.31±0.63 versus 1.10±0.01, p=0.0001) (Fig. 1).

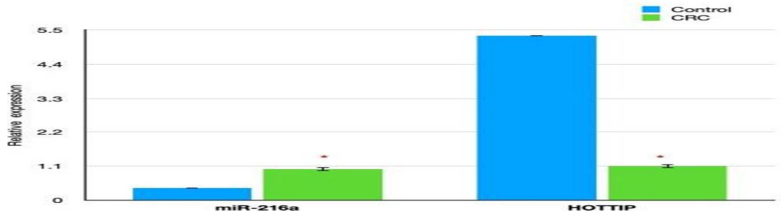

Figure 1: miR-216a and HOTTIP relative expressions level among the studied group

3.3. Relationship between HOTTIP and miR-216a, demographic and clino-pathological data in colorectal cancer group

Data represented in Table 2 showed significant statistical differences between miR-216a of CRC patients with regard to the sites of tumors (Transverse+ flexures) (p=0.020), ulcers (p=0.004), wall thickening (p=0.043), and liver metastasis (p=0.001) within CT Picture, while the other parameters were non-significant. HOTTIP expression level exhibited statistical significance with regard to the sites of anatomy of CRC patients in (Ceacum+Ascending) colon (p=0.03) and (Rectum+Sigmoid) colon (p=0.038), on the other side, mass, ulcer, hyperemia, mass lesion, wall thickening, Regional LNs, liver metastasis, and the types of the tumor (adenocarcinoma and mucoid) had no statistical significance with long non coding HOTTIP expression.

Table 2: Association between HOTIIP and miR-216a, demographic and clino-pathological data in CRC group

Parameters	miR-216a	p-value	HOTTIP	P- value
Age (Years)	0.39±0.069	0.8[a]	5.31±0.63	0.67
Gender				
Female	0.59±0.15	.116 [b]	6.59±1.45	0.19
Male	0.31±0.06		4.59±0.60	
Site of anatomy				
Ceacum+Ascending	0.19±0.76	0.083[a]	6.18±1.46	**0.03***[a]
Transverse+Flexures Rectum+Sigmoid	0.42±0.13	**0.020***[b]	4.18±0.49	0.77[b]
	0.46± 0.10	0.60[c]	5.57±1.04	**0.038***[c]
Colonoscopy Picture				
Mass				
Yes	0.09±0.044	0.72	5.63±0.71	0.85
No	.41±0.072		4.71±1.22	
Ulcer				
Yes	0.33±0.14	**0.004***	5.52±0.72	0.445
No	0.42±0.07		4.32±1.19	
Hyperemia		0.53		
Yes	0.35±0.06		5.45±0.64	0.086
No	0.55±0.19		4.29±1.05	
CT Picture				
Mass Lesion	0.24±0.08	0.18	5.77±0.80	0.108
Yes	0.35±0.08		4.02±0.70	
No				
Wall Thickening	0.24±0.071	**0.043***	5.34±1.09	0.96
Yes	0.28±0.25		5.33±0.53	
No		0.87		
Regional LNs	0.45±0.081		5.74±0.81	
Yes	0.15±0.049		4.30±0.85	0.35
No		**0.001***		
Liver Mets	0.39±0.08		5.40±0.66	
Yes	0.40±0.12		4.63±0.96	0.331
No				
Tumor Type				
Adenocarcinoma	0.41±0.07	0.8	5.13±0.71	0.779
Mucoid	0.33±0.16		6.16±1.32	

* Data exhibit statistical significance at (P≤0.05).

[a] Ceacum+Ascending &Transverse+Flexures; b Ceacum+Ascending & Rectum+Sigmoid; c Transverse+Flexures and Rectum+Sigmoid

3.4. The diagnostic Performances of miR-216a and HOTTIP expression in CRC patients

The ROC analysis curves and the corresponding area under the curve were calculated for providing the diagnostic performances of miR-216a and HOTTIP to discriminate CRC patients (Table 3). ROC curve for miR-216a indicated reliable differentiation between normal and CRC tumor tissues at a cut-off value of 0.69 (AUC =0.87, sensitivity about 87.7% and specificity 90.8%). Regarding HOTTIP, it discriminated CRC from healthy individuals with best cut-off value = 5.6 (AUC= 0.94, with sensitivity= 94.7% and specificity = 98.6%) (Fig. 2).

Table 3: Diagnostic and prognostic performances of miR-216a and HOTTIP

Parameter	AUC	Cut-off value	Sensitivity	Specificity	95% C.I	Accuracy	p-value
miR-216a	0.87*	0.69	87.7%	90.8%	0.76 - 0.94	89.25%	<0.0001
HOTTIP	0.94*	5.6	94.7%	98.6%	0.88 - 0.98	96.65%	<0.0001

* Data exhibit statistical significance at (P≤0.05). AUC, area under the curve

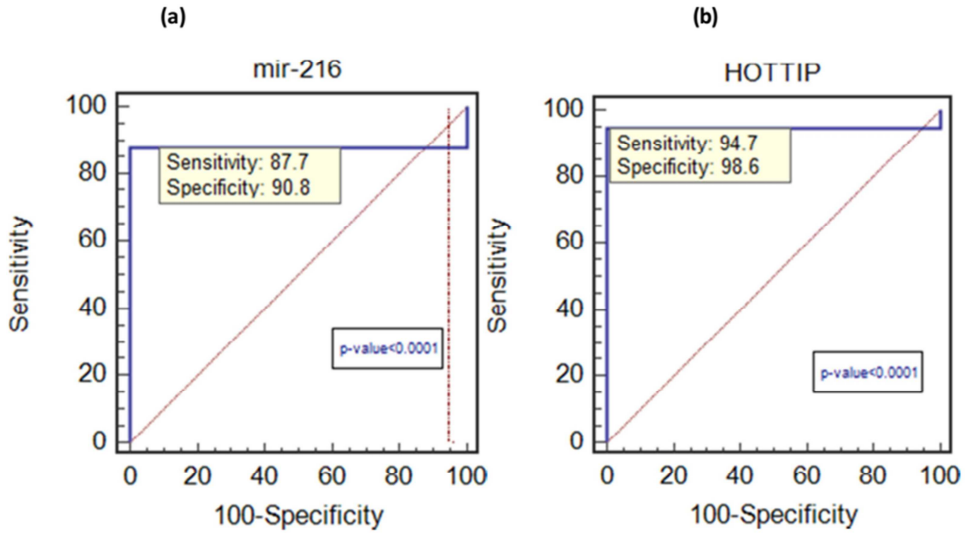

Figure 2: ROC Curve analysis for miR-216a and HOTTIP among CRC patients

3.5. Associations between HOTTIP rs3807598 C/G genotypes and the risk of colorectal cancer

In accordance with Hardy–Weinberg equilibrium (P= 0.38) the genotype and allele distributions of HOTTIP SNP (rs3807598) in CRC patients and controls are shown in (Table 4) the findings demonstrated that GC and GG genotypes are significantly more prevalent in CRC patients compared to healthy controls. We found that the prevalence of the rs3807598 GG homozygous mutant genotype was considerably higher in colorectal cancer than in controls (26.3% versus 6%, respectively, P= 0.001).

Upon comparing the distribution of rs3807598 genotypes and alleles between colorectal cancer and healthy controls, we got a significant difference with higher mutant genotype (GG) and allele (G) prevalence in colorectal cancer than controls (26.3% versus 6%, respectively, for genotypes, p=0.001) and (54% versus 23%, respectively, for alleles, p= 0.001). C allele of HOTTIP rs3807598 in the CRC group were significantly lower than those in the control group (60 versus 77, p=0.0001), while the G allele in the CRC group were significantly higher than the control group (54 versus 23, p=0.0001).

Table 4: Genotypes and Allelic distribution of HOTTIP rs3807598 C/G in Colorectal cancer and control group

(rs3807598)	CRC	Control	p-value
Genotypes			
CC	18(31.6%)	30(60%)	**0.001***
CG	24(42.1%)	17(34%)	**0.048***
GG	15(26.3%)	3(6%)	**0.001***
Alleles			
C	60	77	**0.0001***
G	54	23	**0.0001***

Hardy-Weinberg equilibrium; X2 = 0.77, P = 0.380. * Data exhibit statistical significance at (P≤0.05).

3.6. Association between HOTTIP genotypes versus the type and sites of tumors among CRC group

Table (5) indicated significant statistical difference with regard to CG genotype and G allele and the different sites of tumor of CRC in (Ceacum+Ascending), (Transverse+Flexures), and (Rectum+Sigmoid) with p=0.001. On the other hand, CC and GG non-significant.

Table 5: Association between HOTTIP genotypes with respect to type and sites of CRC

HOTTIP genotype	Adeno-carcinoma	Mucoid	p-value	Ceacum+ Ascending	Transverse+ Flexures	Rectum+ Sigmoid	p-value
Genotypes %)			**(Count**				
CC	17(30%)	1(1.8%)	**<0.05**	6(10.5%)	8(14.2%)	4(7.0%)	0.8
CG	17(29%)	7(12.5%)	**<0.05**	3(5.2%)	3(5.2%)	18(31.5%)	**0.001**
GG	13(23%)	2(3.7%)	**<0.05**	3(5.2%)	5(9%)	7(12.2%)	0.08
C	42(36.8%)	9(7.8%)	**0.001**	15(13.15%)	19(16.6%)	26(22.8%)	0.08
G	52(45.6%)	11(9.8%)	**0.001**	9(7.9%)	13(11.40%)	32(28.2%)	**0.001**

* Data exhibit statistical significance at (P≤0.05)

3.7. Comparison of different genotypes of HOTTIP rs3807598 and laboratory indices of CRC patients

Laboratory parameters such as Platelet, AST, and bilirubin showed statistically significant change with regard to genotypes CC and GG. On the other hand, comparing other genotypes (CC and CG), (CG and GG) with laboratory parameters Hg, Platelets, ALT, AST, Bilirubin, and Albumin were statistically non- significant (Table 6).

Table 6: Genotypes of HOTTIP rs3807598 and laboratory indices of CRC patients

Parameter	Genotypes in CRC patients			
	HOTTIP rs3807598			
	CC	CG	GG	p-value
Age	54.17±2.75	51.08±2.43	50.47±3.27	0.313a 0.441b 0.984c
Gender				
Male	13(22.83%)	17(29.82%)	9(15.78%)	0.474
Female	5(8.77%)	7(12.28%)	6(10.52%)	
Hg (g/dL)	11.02±0.80	11.30±0.56	10.47±0.63	0.44[a] 0.19[b] 0.34[c]
Platelets	240.6±24.9	280.6±21.48	290.60±22.46	0.57[a] **0.05**[b] 0.64[c]
ALT	20.88±1.85	23.29±2.02	21.53±2.56	0.29[a] 0.28[b] 0.92[c]
AST	25.5±1.75	25.7±1.59	26.53±2.93	0.98[a] **0.04**[b] 0.039[c]
Bilirubin	0.96±0.06	0.71±0.07	0.73±0.08	0.057[a] **0.04**[b] 0.45[c]
Albumin	3.96±0.15	4.47±0.21	3.86±0.16	0.063[a] 0.61[b] 0.06[c]

Data exhibit statistical significance at (P≤0.05).
a between CC and CG; b between CC and GG; c between CG and GG.

3.8. Association between HOTTIP and miR-216a expressions profile among HOTTIP genotypes

MiR-216a was highly expressed in genotype GG with a mean of 0.51, followed by CG genotype with a mean of 0.49. While, the expression level of HOTTIP was highest with genotype GG with a mean of 7.11 (Fig 3).

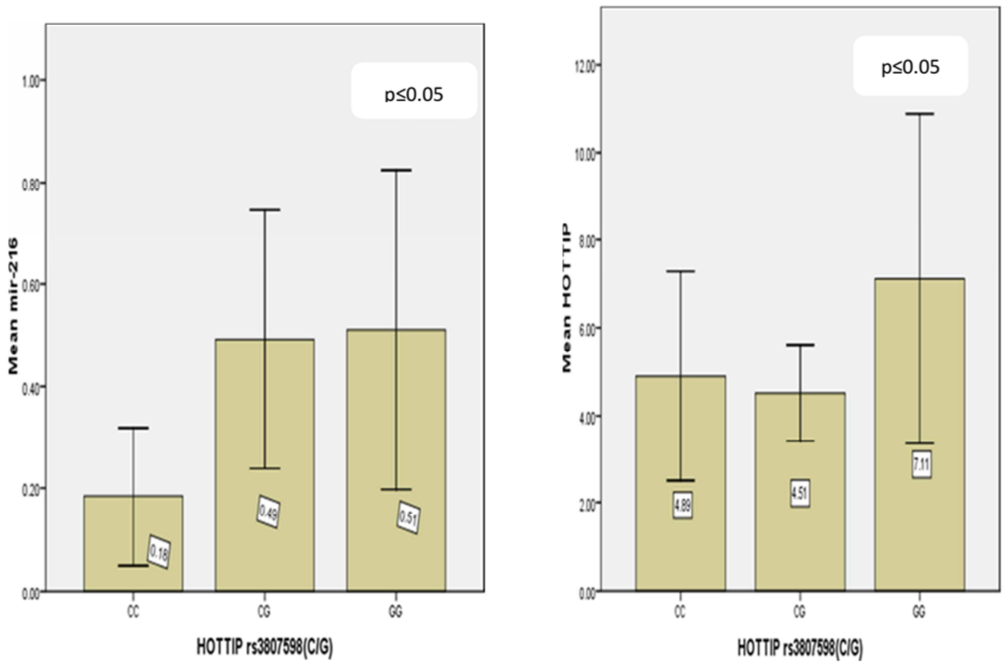

Figure 3: Expression levels of miR-216a and HOTTIP according to the genotypes CC, CG and GG

4. Discussion

In low- and middle-income nations, the prevalence of colorectal cancer has been steadily rising over the last few decades. Colorectal cancer, is mostly brought on by colorectal adenomas and a major global cause of cancer-related mortality [14],[15]. Because of the lack of early diagnosis, CRC is a heterogeneous disorder that poses a clinical difficulty [16]. Consequently, non-invasive prospective biomarkers with strong diagnostic and prognostic capabilities are highly desired. This study assessed the genetic relationships between CRC susceptibility and HOTTIP SNP rs3807598, along with examining the diagnostic performance of non-coding RNAs HOTTIP and miR-216a expressions among CRC patients.

MicroRNAs participate in multiple types of cancer, such as pancreatic cancer, breast cancer, and colorectal cancer [17]. Because of their important function in CRC, microRNAs have been found to represent both viable therapeutic targets and diagnostic and prognostic biomarkers of the disease. The progression of malignant tumors was significantly aided by the malfunction and dysregulation of miRNAs. MiRNA has a tumor- suppressive or oncogenic impact [18]. MiR-216a is a tumor suppressor that regulates many target mRNAs in various cancers, making it one of the potential miRNA therapeutic targets [16]. Herein, the current study reported significant down regulation in miR- 216a among CRC patients versus normal individuals. These results were in line with Wang [4], who indicated that down regulation of miR-216a-3p was strongly related to the development of colorectal cancer in tissues and cell lines, while knockdown of COX-2 or ALOX5, which promote CRC cell.

Cellular transcripts are largely composed of long non-coding RNAs (lncRNAs), which are now known to be crucial components of several biological processes. They have attracted a lot of attention recently since it is believed that they are engaged in both developmental stages. Because of their unique expression and functional diversity across a range of malignancies, long noncoding RNAs have potential use in the diagnosis, prognosis, and treatment of cancer. Studies have shown that due of their high specificity and accuracy, lncRNAs may serve as cancer biomarkers. It is possible to extract lncRNAs from body fluids, tissues, and cells without undergoing intrusive techniques. They can then be employed as primary or secondary biomarkers to increase the precision of a diagnosis or prognosis [20]**.** LongncRNAs can regulate expression of genes through their interaction domains for DNA, mRNAs, miRNAs, and proteins, whereas miRNAs can mediate posttranscriptional regulation of gene expression through translational repression or mRNA destruction [21].

Abnormal expression of LncRNAs HOTTIP participates in the development of a several types of malignancies [7]. HOTTIP binds to WDR5- MLL complexes and directs them to 5′ HOXA locus, where they produce a large domain of H3K4me3 and activate transcription. The ectopic expression of HOTTIP reduced invasion, migration, proliferation, and survival of pancreatic cancer cells and By activating Wnt/β-catenin, the overexpression of HOTTIP may enhance

osteosarcoma cell proliferation and progression of cell cycle. In CRC patients, elevated HOTTIP expression is highly expressed in colorectal tissues relative to surrounding normal tissues, and it is intricately related to clinical stage, distant metastasis, and tumour size. [9]. Regarding the expression level of serum HOTTIP, results denoted up regulation of HOTTIP level in CRC patients as compared to healthy group (5.31 vs 1.10, $p< 0.0001$). These results concurred with the study of Liu [22] who reported that the expression of HOTTIP was significantly higher in colorectal cancer (CRC) in comparison to adjacent normal tissues and the level of expression was higher in patients with larger tumours, advanced clinical stages, or distant metastases. Additionally, up regulation of HOTTIP leads to the enhancement of CRC cell growth by inhibiting p21 expression, a cyclin-dependent kinase inhibitor (CKI) which controls cell cycle progression at the G1 and S phases by acting as a checkpoint regulator. Moreover, Liu [23] revealed that by specifically targeting the glucocorticoid-inducible kinase 1 (SGK1) gene, HOTTIP Knock down in HCT-116 and SW620 cell lines promoted apoptosis and inhibited CRC cell growth. Additionally, it suppresses the expression of GSK3β, β-catenin, vimentin, matrix metalloproteinase 7 (MMP-7) and cellular myelocytomatosis oncogene (C-MYC). As a result, E-cadherin is up-regulated.

In parallel with Lian [24] and Ali Akbar-Esfahani [9], comparing the expression level of HOTTIP with the demographic and clinopathological results demonstrating that there were no significant correlations between the dysregulation of HOTTIP expression level versus the gender, age, tumor lymph nodes, or metastasis. Also, the current laboratory data indicated that the Hg levels in all CRC patients decreased significantly in compared to control group. Similar studies by Sawayama [25] and Shaker [26] were consistent with these results, in which the anemia with lower Hg level is a common symptom of CRC. According to the tumor site, previous studies demonstrated that tumor location of CRC affect the expression of miRNAs [27]. In accordance, the current results revealed association between clino- pathological parameters and miR-216a in CRC group, lower miR-216a levels were estimated in cancer that affect Ceacum + Ascending colon as compared with Rectum + Sigmoid. HOTTIP has several SNPs that are predicted to change its function or expression [9]. In this study, the genotype and allele distribution of HOTTIP SNP (rs3807598) were associated with CRC. Results exhibited that the genotypes GC and GG were significantly elevated in CRC patients versus healthy control. Similar studies have found that HOTTIP polymorphisms rs3807598, rs17427960, rs2067087 were significantly associated with CRC risk [28]. Furthermore, the ROC curve analysis exhibited the diagnostic value of HOTTIP with high sensitivity and specificity (94.7% and 98.6% respectively). Also Ali Akbar-Esfahani [7] described HOTTIP as a diagnostic biomarker with sensetivity 76% and specificity 82% and AUC= 0.775 in Iranian patients.

5. Conclusions

In conclusions, the current study highlighted notable significant alterations in miR-216a and HOTTIP expression levels in CRC patients versus heathy subjects. Gene polymorphism HOTIP rs3807598 (C:G) is linked to colorectal cancer. Also, individuals with the GG genotype had greater expression patterns for miR-216a and HOTTIP. Additionally, both serum miR-216a and HOTTIP expression levels have respective diagnostic capabilities and could potentially function as diagnostic biomarkers among CRC patients.

6. Conflicts of interest

"There are no conflicts to declare".

7. References

1. Oh, H. H.; & Joo, Y. E. Novel biomarkers for the diagnosis and prognosis of colorectal cancer. Intestinal research, **2020**, 18(2), 168–183.
2. Sawicki, T.; Ruszkowska, M.; Danielewicz, A.; Niedźwiedzka, E.; Arłukowicz, T.; & Przybyłowicz, K. E. A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. Cancers, **2021**, 13(9), 2025.
3. Ogunwobi, O. O.; Mahmood, F.; & Akingboye, A. Biomarkers in Colorectal Cancer: Current Research and Future Prospects. International journal of molecular sciences, **2020**, 21(15), 5311.
4. Wang, D.; Li, Y.; Zhang, C.; Li, X.; & Yu, J. MiR-216a-3p inhibits colorectal cancer cell proliferation through direct targeting COX-2 and ALOX5. Journal of cellular biochemistry, **2018**, 119(2), 1755–1766.
5. Nemeth. K.; Bayraktar, R.;Ferracin, M.; Calin, GA. Non-coding RNAs in disease: from mechanisms to therapeutics. *Nat Rev Genet*, **2023**, 25(3), 211-232.
6. Singh, A. P.; Luo, H.; Matur, M.; Eshelman, M. A.; Hamamoto, K.; Sharma, A.; Lesperance, J.; & Huang, S. A coordinated function of lncRNA HOTTIP and miRNA-196b underpinning leukemogenesis by targeting FAS signaling. Oncogene, **2022**, 41(5), 718–731.
7. Akbar-Esfahani, A. S.; Karimipoor, M.; Bahreini, F.; Soltania, A. R.; Aletaha, N.; & Mahdavinezhad, A. Diagnostic Value of Plasma Long Non-coding RNA HOTTIP as a Non- invasive Biomarker for Colorectal Cancer (A Case- Control Study). International journal of molecular and cellular medicine, **2019**, 8(4), 240–247.
8. Abdi, E.; Latifi-Navid, S.; & Latifi-Navid, H. Long noncoding RNA polymorphisms and colorectal cancer risk: Progression and future perspectives. Environmental and molecular mutagenesis, **2022**, 63(2), 98–112.
9. Ghafouri-Fard, S.; Dashti, S.; & Taheri, M. The HOTTIP (HOXA transcript at the distal tip) lncRNA: Review of oncogenic roles in human. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, **2020**, 127, 110158.
10. Peng, F.; Shi, X..; Meng, Y.; Dong, B.; Xu, G.; Hou, T.; Liu, T. Long non-coding RNA HOTTIP is upregulated in

renal cell carcinoma and regulates cell growth and apoptosis by epigenetically silencing of LATS2. Biomedicine & Pharmacotherapy, **2018**,105, 1133–1140.
11. Peng, Q.; Shen, Y.; Zhao, P.; Cheng, M.; Zhu, Y.; & Xu, B. Biomarker roles identification of miR-106 family for predicting the risk and poor survival of colorectal cancer. BMC cancer, **2020**, 20(1), 506.
12. Roscigno, G.; Cirella, A.; Affinito, A.; Quintavalle, C.; Scognamiglio, I.; Palma, F.; Ingenito, F.; Nuzzo, S.; De Micco, F.; Cuccuru, A.; Thomas, R.; & Condorelli, G. miR-216a Acts as a Negative Regulator of Breast Cancer by Modulating Stemness Properties and Tumor Microenvironment. International journal of molecular sciences, **2020**, 21(7), 2313.
13. Zeng, X.; Liu, Y.; Zhu, H.; Chen, D.; & Hu, W. Downregulation of miR-216a-5p by long noncoding RNA PVT1 suppresses colorectal cancer progression via modulation of YBX1 expression. Cancer management and research, **2019**, 11, 6981–6993.
14. Dutta, A.; Pratiti, R.; Kalantary, A.; Aboulian, A.; & Shekherdimian, S. Colorectal Cancer: A Systematic Review of the Current Situation and Screening in North and Central Asian Countries. Cureus, **2023**, 15(1), e33424.
15. Chen, X.; Liu, Y.; Zhang, Q.; Liu, B.; Cheng, Y.; Zhang, Y.; Sun, Y.; Liu, J.; & Gen, H. Exosomal Long Non-coding RNA HOTTIP Increases Resistance of Colorectal Cancer Cells to Mitomycin via Impairing MiR-214-Mediated Degradation of KPNA3. Frontiers in cell and developmental biology, **2021**, 8, 582723.
16. Wang, W.; Kandimalla, R.; Huang, H.; Zhu, L.; Li, Y.; Gao, F. Molecular subtyping of colorectal cancer: recent progress, new challenges and emerging opportunities. Semin. Cancer Biol, **2019**, 55, 37–52.
17. Zhang, N.; Hu, X..; Du, Y.; & Du, J. The role of miRNAs in colorectal cancer progression and chemoradiotherapy. Biomedicine& pharmacotherapy = Biomedecine & pharmacotherapie, **2021**, 134, 111099.
18. Shaker, O. G.; Ayeldeen, G.; & Abdelhamid, A.
M. Circulating microRNA-944 and its target gene EPHA7 as a potential biomarker for colorectal cancer. Archives of physiology and biochemistry, **2022**, 128(5), 1181–1187.
19. Zhang D.; Zhao L.; Shen Q. Down-regulation of KIAA1199/CEMIP by miR-216a suppresses tumor invasion and metastasis in colorectal cancer. Int J Cancer, **2017**, 140,2298–2309.
20. Qian, Y.; Shi, L.; & Luo, Z. Long Non-coding RNAs in Cancer: Implications for Diagnosis, Prognosis, and Therapy. Frontiers in medicine, 2020, 7, 612393.
21. Grillone , K.; Riillo, C.; Scionti, F.; Rocca, R.; Tradigo, G.; Guzzi, P. H.; Alcaro, S.; Di Martino, M. T.; Tagliaferri, P.; & Tassone, P. Non-coding RNAs in cancer: platforms and strategies for investigating the genomic "dark matter". Journal of experimental & clinical cancer research : CR, 2020, 39(1), 117.
22. Liu, T.; Wang, H.; Yu, H., Bi, M.; Yan, Z.; Hong, S.; & Li, S. The Long Non-coding RNA HOTTIP Is Highly Expressed in Colorectal Cancer and Enhances Cell Proliferation and Invasion. Molecular therapy. Nucleic acids, 2020, 19, 612–618.
23. Liu, T.; Yu, T.; Hu, H.; & He, K. Knockdown of the long non-coding RNA HOTTIP inhibits colorectal cancer cell proliferation and migration and induces apoptosis by targeting SGK1. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 2018, 98, 286–296.
24. Lian, Y.; Ding, J.; Zhang, Z.; Shi, Y.; Zhu, Y.; Li, J.; Peng, P.; Wang, J.; Fan, Y.; De, W.; & Wang, K. The long noncoding RNA HOXA transcript at the distal tip promotes colorectal cancer growth partially via silencing of p21 expression. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, 2016, 37(6), 7431–7440.
25. Sawayama, H.; Miyamoto, Y.; Hiyoshi, Y.; Shimokawa, M.; Kato, R.; Akiyama, T.; Sakamoto, Y.; Daitoku, N.; Yoshida, N.; & Baba, H. Preoperative transferrin level is a novel prognostic marker for colorectal cancer. Annals of gastroenterological surgery, 2021, 5(2), 243–251.
26. Shaker, O. G.; Ali, M. A.; Ahmed, T. I.; Zaki, O. M.; Ali, D. Y.; Hassan, E. A.; Hemeda, N. F.; & AbdelHafez, M. N. Association between LINC00657 and miR-106a serum expression levels and susceptibility to colorectal cancer, adenomatous polyposis, and ulcerative colitis in Egyptian population. IUBMB life, 2019, 71(9), 1322–1335.
27. Slattery, M. L.; Wolff, E.; Hoffman, M. D.; Pellatt, D. F.; Milash, B. MicroRNAs and colon and rectal cancer: differential expression by tumor location and subtype. Genes, Chromosomes Cancer, 2011, 50(3), 196–206.
28. Lv, Z.; Xu, Q.; Sun, L. Four novel polymorphisms in long non-coding RNA HOTTIP are associated with the risk and prognosis of colorectal cancer. Biosci Rep. 2019;39(5):BSR20180573.