Med. J. Cairo Univ., Vol. 90, No. 8, December: 2767-2775, 2022
www.medicaljournalofcairouniversity.net

Association of Serum Cyclin D1 and Variations of MIR 196A2 and Deleted in Colorectal Cancer (DCC) Genes with Colorectal Cancer

NORA M. SELIM, M.D.*; IMAN A. MANDOUR, M.D.*; RIHAM El-SAYED, M.D.*;
EHSAN EL-GHONEIMY, M.D.**; MAYSA I. FARGHALY, M.D.*; RASHA M. ABDEL SAMIE, M.D.*** and YASMIN T. ELSHEWY, M.D.*

The Departments of Clinical & Chemical Pathology, Medical Oncology** and Internal Medicine***, Faculty of Medicine, Cairo University*

Abstract

Background: Colorectal Cancer is the 5th common malignant tumor among Egyptians representing 5% of the total cancers according to National Cancer Registry 2013.

Aim of Study: To evaluate association of serum level of Cyclin D1 and variants in MicroRNA196a-2 (miR196A2) rs11614913 and Deleted in colorectal cancer (DCC) rs714 genes with the risk of colorectal cancer and its progression.

Patients and Methods: A case-control study was done on 100 colorectal cancer (CRC) patients and 60 healthy controls were enrolled and were genotyped for miR196A2 rs11614913 gene variation using the real time-Taq Man assay and DCC rs714 gene variation using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Serum levels of Cyclin D1 were measured by Enzyme linked immunosorbant assay (ELSA).

Results: Pathogenic variant G of DCC was associated with an increased risk of colorectal cancer compared to A variant (p<0.001). Also, G variant (GG and GA genotypes) was associated with increased risk of distant metastasis in patients of CRC compared to DCC A variant (p=0.014). There was a significant association between increased serum level of Cyclin D 1 and the risk of colorectal cancer (p=0.001) with cut-off value >3.4ng/mL. Pathogenic variant C of miR196A2 was not significantly associated with increased risk of colorectal cancer (p=0.766) but showed a significant increase in tumor progression (p=0.013).

Conclusion: Pathogenic variant G of DCC and increased serum level of Cyclin D1 are predictive factors of sporadic colorectal cancer among Egyptian patients. C variant of miR196A2 can be used as prognostic rather than diagnostic markers of CRC.

Key Words: *Colorectal cancer – microRNA – Genes – DCC – Polymorphism – Single Nucleotide – Cyclin D1 miR196A2 rs11614913 – DCC rs714.*

Correspondence to: Dr. Nora M. Selim,
E-Mail: nora.selim@hotmail.com.

Introduction

COLORECTAL cancer (CRC) is a leading cause of cancer death globally and is considered the third cause of cancer mortality in the United States among adults, younger than 50 years of age **[1]**. CRC represents the fifth most common malignant tumor accounting for 5% of the total estimated cancers among Egyptian populations. CRC rates recorded in Egyptian studies, in patients under the age of 40 years, were higher than that reported in the West **[2]**.

The mechanism of carcinogenesis in CRC has not yet been fully elucidated **[3]**. It may be caused by an interplay between genetic and environmental factors **[4]**. Advances in molecular pathology of cancer have also identified key signaling pathways implicated in the pathogenesis and progression of colorectal cancer **[5]**. Moreover, recent genome wide association (GWA) have revealed that some single nucleotide polymorphisms (SNPs) in the microRNA genes could change the expression and/or the maturation of miRNA and may be linked to cancer predisposition and progression **[6]**. MicroRNAs are a big class of small noncoding RNAs, which take part in different biological processes by contributing to translational and transcriptional regulation and may control tumor suppressor genes or oncogenes **[7,8]**. Tumorigenesis and cancer progression dysfunction are partly affected by microRNAs through translational repression or degradation of target mRNAs **[9]**. Several case-control studies were conducted to evaluate the relationship between miR196A2 C>T variations and the risk of colorectal cancer, however, the results were conflicting **[10,11]**. The rs11614913 could affect the mature miR-196A2 expression levels, thus influencing the expression of its target gene, which

in turn may have an impact on the regulation of carcinogenesis [12]. The T allele of miR-196A2 rs11614913 was found to be linked to an increased risk of CRC occurrence among Chinese populations [13]. The rs11614913 may affect miRNA expression in CRC patient, demonstrating a close association with tissue invasion, differentiation, staging, lymph nodes metastasis and thus has an implication as a diagnostic and prognostic biomarker for CRC patients [14].

Variations in tumor suppressor genes also contribute to carcinogenesis [15]. Deleted in colorectal cancer (DCC) gene, located on chromosome 18q21.2, is considered a tumor-suppressor gene. It encodes for a trans-membrane receptor that binds to nitrin-1 as ligand. Those receptors are functional dependence receptors that induce apoptosis in the absence of their ligand but inhibit apoptosis when bound to netrin-1 [16]. A study by Djansugurova et al. demonstrated that the G variant of DCC rs714 was associated with reduced expression of DCC with subsequent increased risk of CRC [17].

CRC is associated with overproduction of Cyclin D1 in various studies [18,19,20]. Cyclin D1/ Cyclin-dependent kinase 4 (Cdk4)-complex phosphorylates Retinoblastoma protein (pRb) and hence regulates the switch of the cycle from the Gap 1 (G1) phase to the synthesis phase (S1) [20]. Overproduction of Cyclin D 1 and CdK will subsequently disrupt the normal control of the cell cycle resulting in enhanced cellular proliferation and thus contributing to development of malignancy and its progression [21,22].

Cyclin D1 over production has been observed in approximately 40-70% of colorectal tumors and is considered a significant factor for prognosis and prediction of survival of CRC patients [22,23]. It has also been associated with advanced disease stage, as elevated concentration was detected in 66.7% of patients with stage IV compared to 26.7% in stage I to III patients [24].

The aim of this study was to evaluate the association between the risk of colorectal cancer and serum levels of Cyclin D1 and variations in rs714 and rs11614913 and to correlate them with tumor site, stage, histopathological grade, lymph node involvement and distant metastasis.

Patients and Methods

This case-control study was conducted on 100 colorectal cancer subjects. They were retrospectively recruited from Internal Medicine Department and Oncology Department, Kasr El-Ainy Hospital, Cairo University, during the period from December 2014 to June 2018. The study was carried out after approval by the Research Ethics Committee of Cairo University Hospital and in accordance with the Declaration of Helsinki [25]. A written informed consent was obtained from all subjects enrolled in the present study. Sixty apparently healthy individuals who were age- and sex- matched were included as controls. Inclusion criteria for patients: Patients with cancer colon before or after surgical removal of the tumor, patients with cancer rectum before surgical removal of the tumor, patients with colorectal cancer with distant metastasis at first presentation. Their age ranged between 17-70 years. Patients with concomitant malignancies were excluded from the study.

Laboratory analysis was carried out in the Chemical Pathology Department, Molecular Unit, Cairo University. All patients were subjected to full history taking, clinical examination and diagnosis of malignant colorectal lesions was established by colonoscopy and biopsy, done at the Gastroenterology and Endoscopy Unit of Kasr El-Ainy Hospital. Histopathological assessment of tumor histological type and grade was performed. The Tumor Node Metastases (TNM) staging system of the American Joint Committee on Cancer (AJCC) was applied for staging the CRC patients [26]. CT Abdomen and Chest with oral and intravenous contrast was performed for all patients after diagnosis of colorectal cancer by colonoscopy. Carcino- Embryonic Antigen (CEA) was assayed by chemiluminescence immunoassay technique.

Assessment of serum Cyclin D1 and Genotyping assay:

All participants were subjected to:

A- Specimen collection and storage:

Five ml of venous blood were aspirated under aseptic conditions and partitioned as follows: (A) Three milliliter (3ml) were collected in a plain sterile vacutainer. Blood samples were left to clot at room temperature and then centrifuged for 5 minutes for serum separation and kept at –20ºC until time of assay of Cyclin D 1. (B) Two milliliters (2ml) of venous blood were drawn into a sterile EDTA vacutainer tube and stored at –20ºC till time of doing the genotyping technique.

B- Analysis of serum Cyclin D1:

Cyclin D 1 was measured by Human Cyclin D 1 ELISA kit supplied by Abbexa (Cambridge Science Park, Cambridge, CB4 0EY, UK) which employs a double-antibody sandwich enzyme-linked immunosorbent assay.

C- *Genotyping:*

Extraction of genomic DNA was performed using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) by silica-gel spin columns **[27]**.

After DNA extraction, the extracted DNA was evaluated qualitatively and quantitatively by spectrophotometric instrument (Quawell UV-Vis spectrophotometer Q5000):

Qualitative evaluation by the DNA/protein ratio absorbance 260/280 was done. All samples ranged (2.2-3.6) knowing that accepted range should exceed 1.8.

Quantitative evaluation by the same instrument was performed and all samples measured (22-34ng/ µL) knowing that accepted value should exceed 20ng/ µL.

Analysis of DCC rs714 variation using Polymerase Chain Reaction (PCR) Followed by Restriction Fragment Length Polymorphism (RFLP) was performed as described by Malik et al., **[28]**. DNA amplification was done in a Gradient thermal cycler. Specific oligonucleotide primers were supplied by Bio Basic Canada Inc (Markham, Ontario, Canada) as follows: Forward primer 5'-TGCACCATGC-TGAAGATTGT -3' and Reverse primer 5' AGT-ACAACACAAGGTATGTG-3' **[29]**. Digestion of PCR product with Msp 1 (ball) restriction endonuclease enzyme supplied by Sib Enzyme (Sib Enzyme Ltd., Timakova, Novosibirsk, Russia). The (A) allele remained undigested 396-bp fragment, whereas the (G) allele was expected to yield 256- and 140-bp fragments. According to the interpretation of Malik et al., **[28]** the RFLP procedure yielded one of 3 possible genotypes: AA/ GA/ GG; (396) fragment site (bp) for AA, (396, 256, 140) for heterozygous GA, and (256,140) for homozygous GG respectively.

Real-time PCR allelic discrimination of rs 11614913 was held using Taq-Man SNV Genotyping Assays and done on Step-One (Applied Biosystems, Life Technologies Corporation, USA), to define rs 11614913 (Fig. 1) as described by Hezova et al. **[30]**.

Statistical analysis:

The SPSS computer software, version 10.0 (Chicago, IL, USA) was applied for analysis of data. For nonparametric variables, analysis of Quantitative data was performed using median and range. However, mean ± SD was used for analysis of parametric variables. Whereas qualitative variables data was summarized as frequency and percentage. Student's *t*-test was used for comparison between groups. Analysis of variance was applied followed by a post hoc test, while non-parametric quantitative variables were compared using the Mann Whitney test and Kruskal Wallis test. Comparison of qualitative variables was performed by the Chi square or Fisher's Exact test. Spearman correlation coefficients were calculated to signify the association between different quantitative variables. The association between cancer colon and both miR196A2 genotype and DCC genotype was explored and quantified by odds ratio (OR). The discriminating ability of Cyclin D1 to differentiate cases with cancer colon from those without the disease was determined using Receiver operating characteristic (ROC) curve analysis.

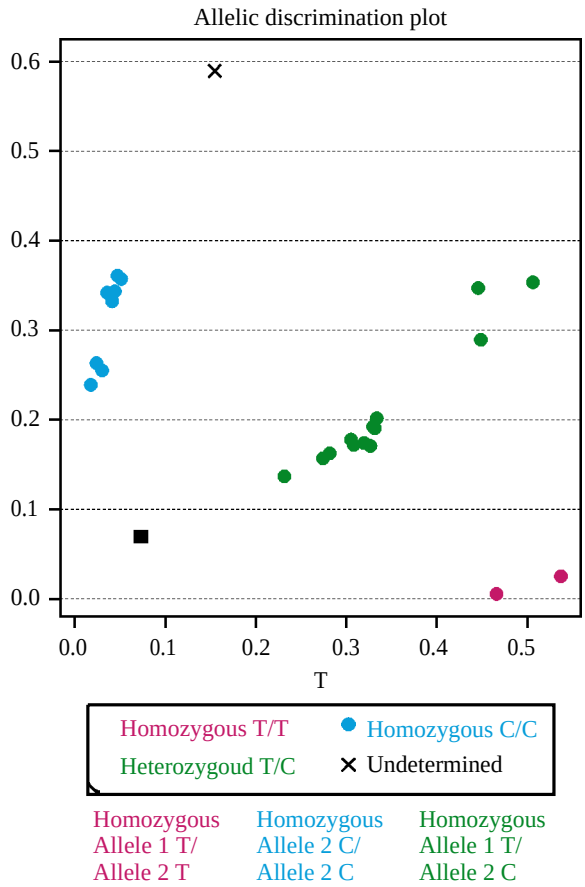

Fig. (1): Allelic discrimination plot to differentiate C/T alleles of miR196A2 gene.

Results

The present study showed the mean age at presentation was 48.3 ± 13.3 years with a male preponderance among the colorectal cancer patients (58% were males as compared to 42% females; male to female ratio=1.4:1; *p*-value=0.014). The demographics and characteristics of all subjects and controls including histopathological features of colonic biopsies obtained from patients with CRC are presented in (Table 1).

Table (1): Demographic data and characteristics of the studied groups and histopathological features of patients.

	Cases (N=100) N (%)	Control (N=60) N (%)	*p*-value
Age (years) (Mean ± SD)	48±13.3	46.3±15.9	0.637
Smoking:			
Current smokers	44 (44%)	25 (41.7%)	0.748
Ex-smokers	4 (4%)	4 (6.7%)	
Non-smokers	52 (52%)	31 (51.7%)	
CIBD:			
Yes	63 (63%)	25 (41.7%)	0.013
No	37 (37%)	35 (58.3%)	
Family history of malignancies:			
Yes	9 (9%)	6 (10%)	0.834
No	91 (91%)	54 (90%)	
TNM stage:			
Stage I	13%	NA	
Stage II	40%		
Stage III	9%		
Stage IV	38%		
Distant Metastasis (Stage IV):			
Present	38%	NA	
Absent	62%		
Lymph node involvement:			
Positive	11%	NA	
Negative	89%		
Histopathological grade:			
Well-differentiated	29%	NA	
Moderately differentiated	55%		
Poorly differentiated	16%		

CIBD: Chronic inflammatory bowel disease.
p-value <0.05 is considered significant.
N: Number. %: Frequency.

Our study revealed a statistically significant increase in level of Cyclin D1 in cases with CRC (median level=7.9ng/mL with an interquartile range: (3.30-76.80) as compared to the control group (median levels; 1.3ng/mL with an interquartile range: 0.01-7.90), indicating significant association between increased level of Cyclin D 1 and risk of colorectal cancer (*p*-value 0.001) (Table 2).

Table (2): Comparison of Cyclin D1 serum level among the studied groups.

	Cases (N=100)	Control (N=60)	*p*-value
Cyclin D1 (ng/ml):			
Median	7.9	1.3	0.001
(Interquartile Range)	(3.30-76.80)	(0.01-7.90)	

p-value <0.05 is of statistical significant.

Furthermore, cases with family history of malignancies demonstrated a significant increase in Cyclin D1 rather than cases without family history of malignancies (*p*-value=0.042). However, levels of Cyclin D1 did not differ significantly according to the gender, site of tumor, stage of tumor, lymph node involvement, or presence of distant metastasis (*p*-values >0.05).

Receiver operating characteristic (ROC) curve was done to explore the discriminating ability of Cyclin D 1 (ng/ml) in predicting cancer colon. It showed an area under the curve (AUC) of 0.983; 95% sensitivity; 95% specificity; 95% PPV; 95% NPV; *p*-value<0.0001 and high accuracy of 95% with a cut-off point of >3.4ng/mL (Fig. 2).

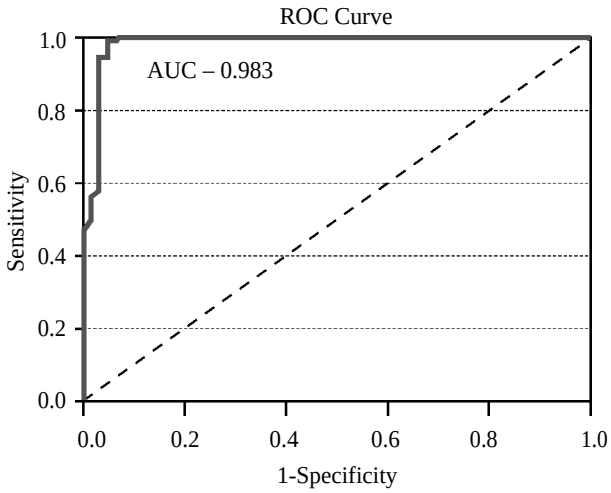

Fig. (2): Receiver operating characteristic (ROC) curve analysis to explore the discriminant ability of Cyclin D1 (ng/ml) in diagnosing cancer colon.

Patients harboring the homozygous pathogenic variant GG for DCC rs714 had 3.1 risk increase for CRC (*p*-value=0.001) (Table 3). Also, The GG genotype of DCC showed a significant increase in cases with distant metastasis compared to DCC AA genotype in patients without distant metastasis (*p*-value=0.014) (Table 4).

Table (3): Genotype frequency in DCC rs714 in studied groups.

Genotype	Cases (n=100) Count (%)	Controls (n=60) Count (%)	*p*-value
Homozygous Mutant (GG)	62 (62%)	12 (20%)	0.001
Heterozygous Mutant (AG)	28 (28%)	42 (70%)	
Wild (AA)	10 (10%)	6 (10%)	

In DCC rs714, A is the wild gene and G is the mutant gene.
N: Number of subjects.
p-value <0.05 is considered significant.

Table (4): Relation between DCC rs714 genotype and incidence of distant metastasis.

	DCC genotype			*p*-value
	GG	AG	AA	
	N (%)	N (%)	N (%)	
Metastasis:				0.014
Yes	29 (46.8)	9 (32.1)	0 (0.01)	
No	33 (53.2)	19 (67.9)	10 (100.0)	

On the other hand, subjects with the homozygous pathogenic variant CC of the miR196A2 rs11614913 who represented 53% of cases and 41.7% of controls and those who were heterozygous CT represented 39% of cases and 48.3% of controls showed no statistical significant difference between studied groups (p-value=0.3 8 1) (Table 5). However, there was a significant increase in miR196A2 rs11614913 pathogenic varians CC and CT in advanced tumor stages compared to the early non-invasive stage (p-value=0.013) (Table 6).

Table (5): MIR196A2 rs11614913 genotyping frequencies in studied groups.

Genotype	Cases (n=100) Count (%)	Controls (n=60) Count (%)	p-value
Homozygous Mutant (CC)	53 (53%)	25 (41.7%)	0.381
Heterozygous Mutant (CT)	39 (39%)	29 (48.3%)	
Wild (TT)	8 (8%)	6 (10%)	

In miR196A2 rs11614913, T is the wild gene and C is the mutant gene. p-value <0.05 is considered significant.

Table (6): Relation between miR196A2 rs11614913 genotypes and tumor stage in colorectal cancer patients.

	MIR196A2 genotype			p-value
	CC 53 (%)	CT 39 (%)	TT 8 (%)	
	N (%)	N (%)	N (%)	
Stage:				
Stage I	11 (29.8)	5 (12.8)	5 (62.5)	0.013
Stage II	36 (67.9)	27 (69.2)	1 (12.5)	
Stage III	6 (11.3)	7 (17.9)	2 (25.0	

- In MIR196A2 rs11614913, T is the wild gene and C is the mutant gene

N: Number of subjects. p-value <0.05 is considered significant.

Discussion

Recent evidence from various studies have emphasized the role of molecular signaling pathways of the cell cycle **[5,21,31]** and gene polymorphisms in the pathogenesis and progression of colorectal cancer. Cyclin D1 overexpression has been reported in around one third or more of CRC cases and was found to be associated with tumor progression, metastasis, and worse prognosis **[18,21]**. Cyclin D 1 acts as a key regulator of cell cycle transition from the G1 to the S phase and its up-regulation can result in enhanced cell proliferation, angiogenesis, and inhibition of apoptosis **[24]** thus promoting carcinogenesis.

Variation in microRNA expression has also been implicated in CRC carcinogenesis **[6,9,11]** with many conflicting reports regarding the association of miR-196A2 C>T polymorphism with the risk of CRC in humans **[11,30,32]**. Multiple genetic alterations in tumor suppressor genes like Deleted in Colorectal Cancer (DCC) gene have also been found to be associated with CRC. The DCC gene influences cell migration, arrest of cell cycle and promotes cell death. It was observed that its expression has been often downregulated or inactivated in different cancers, **[33,34]** with loss of heterozygosity (LOH) at 18q being the most frequently implicated in the pathogenesis of CRC **[35]**. Other gene alterations in DCC, like point mutations, homozygous deletions of the 5' end, various insertions, abnormal genes, decreased or loss of protein and gene expression have also been found to be associated with advanced tumor and metastasis in various cancers **[34]**. Moreover, gene variants of DCC have been linked to increased cancer susceptibility, with the DCCrs714 A>G polymorphism being the most studied and conferring an increased risk of CRC **[35]**.

The present work showed significant prevalence of DCC rs714 GG genotype in cases of colorectal cancer versus the control group (p-value <0.001) {(OR=3.1), 95% CI (0.947-10.149)}. There was also an increased frequency of variant G allele among the cases (76%) as compared to controls (55%) (p-value <0.001) {(OR= 2.591), 95% CI (1.596-4.206). Predominance of the DCC GG genotype among patients with distant metastasis when compared to cases with localized tumor (p-value <0.001) lays emphasis on the potential link of this gene polymorphism with aggressive behavior and rapid progression of CRC.

These results are compatible with a study on an Asian population by Chandra et al., **[34]** who found that DCC rs714 polymorphism was associated with an increased risk of colorectal cancer with significant differences being observed in the frequency of the GG genotype between cases and controls (p-value <0.01). Toma et al., **[29]** observed an overall significantly increased colorectal cancer risk in patients with DCC mutation. Djansugurova et al., **[17]** similarly established an increased risk of CRC with G variant of DCCrs714 (GG and GA genotypes) when compared to AA genotype which was found to have a protective role. It was also reported that the GG genotype was associated with the tumors located in the left colon **[36]**.

Moreover, various studies confirmed that decreased or loss of protein expression of the DCC gene was observed with an increased frequency of advanced clinical stages and was associated with poor differentiation and prognosis in colorectal carcinoma **[37-40]**. On the contrary, a study on a Romanian population showed that the G allele was

coupled with protection from CRC (OR=0.34; 95% CI (0.947-10.149), while the AA genotype (OR= 2.97; 95% CI (1.445-4.205) and A allele {(OR= 2.87), 95% CI (1.732-4.159)} were associated with an enhanced risk for CRC [29].

There had been many conflicting reports regarding the association of miR196A2 C>T polymorphism with the risk of CRC. In the current study, miR196A2 rs11614913 variation was not associated with an elevated colorectal cancer risk and no significant increase or decrease in risk for CRC was observed in subjects who were homozygous or heterozygous for miR196A2 gene polymorphism (OR=1.368, 95% CI: 0.839-2.230).

This was consistent with the results of a study by Shi et al., [41] who demonstrated no association of miR196A2 polymorphism with the risk of colorectal cancer. On the other hand, subgroup analysis based on ethnicity in the latter study, established a significant association between the C allele of miR196A2 C>T polymorphism and an increased susceptibility to CRC when compared to T allele in Asian population. No link was, however, observed in the Caucasian population, thus highlighting the implication of diverse genetic background and lifestyle factors on the susceptibility to the disease. Similar studies by Wang et al., [42] and Parlayan et al., [43] also found no association between miR196A2 polymorphism and CRC risk in Caucasian population.

The results of our study are also in accordance with studies by Chen et al., [35], Hezova et al., [30] and a meta-analysis by Pan et al., [45] which did not demonstrate an association between miR196A2 single nucleotide polymorphisms and risk for CRC. On the contrary, studies by Zhu et al., [46] recognized the CC genotype of miR196A2 rs11614913 as the risk genotype increasing the susceptibility to CRC. Zhan et al., [47] also confirmed an enhanced expression of miR-196A2 in colorectal cancer tissues of patients with the CC or CT genotypes as compared to TT genotype.

The discrepancy in results between studies regarding the probable significance of miR196A2 rs 11614913 in CRC may be attributable to some factors: Such as sample size with a consequent reduced statistical power to assess the association between the polymorphisms and susceptibility to CRC; histological patterns or types of CRC; different ethnicity and genetic variants or different molecular pathological mechanisms that contribute differently to cancer [32,41]. Therefore, further studies with larger sample size and incorporation of gene-gene and gene-environment interactions are warranted to confirm these findings [41,48].

In the present study, increased serum level of Cyclin D1 was associated with increased incidence of colorectal cancer (p-value <0.001) and hence suggesting an oncogenic role of Cyclin D 1 in CRC patients. This agrees with recent studies by Mohamed et al., [49] which demonstrated Cyclin D1 overexpression in around 60% and 56.25% respectively in cases with CRC.

Another important finding in our study was the discriminating ability of Cyclin D1 in diagnosing CRC with a high sensitivity, specificity and diagnostic accuracy (95% sensitivity; 95% specificity; 95% PPV; 95% NPV; 95% accuracy) with a cut-off value of >3.4ng/mL.

However, we found that levels of Cyclin D1 did not differ significantly according to the gender, site of tumor, stage of tumor, lymph node involvement, or presence of distant metastasis (p-values >0.05). On the other hand, a study by Albasri et al., [21] revealed that increased Cyclin D1 expression significantly correlated with tumor differentiation, lymphovascular invasion, lymph node affection as well as evidence of distant metastasis.

Previous studies by Bahnasy et al., [18] and Ogino et al., [50] also found that Cyclin D1 is one of the superior independent indicators of poor prognosis in colorectal cancer patients and is associated with shortened survival. Conversely previous studies by Schernhammer et al., [51] and Forones et al., [52] found no association between Cyclin D1 and the risk of colorectal cancer.

In our study, an increase in the serum levels of Cyclin D1 was observed in CRC cases with family history of malignancies with (p-value <0.001). Likewise, Schernhammer et al., [51] found that overexpression of Cyclin D1 in conjunction with a family history of colorectal cancer presented a considerably greater risk of colorectal cancer (p-value <0.001).

Conclusion:

This study suggests that both DCC rs714 gene variation and Cyclin D1 level are associated with elevated colorectal cancer risk in the studied cohort of the Egyptian population. It is, therefore, necessary to conduct a large-scale population study to elucidate our results. The single nucleotide polymorphism C/T MIR 196-A2 gene is a valuable biomarker in the progression of colorectal cancer; its relation with advanced tumor characteristics should be investigated to predict tumor response to targeted therapies in colorectal cancer patients.

References

1- BHANDARI A., WOODHOUSE M. and GUPTA S.: Colorectal cancer is a leading cause of cancer incidence and mortality among adults younger than 50 years in the USA: A SEER-based analysis with comparison to other young-onset cancers. Journal of Investigative Medicine, 65: 311-315, 2017.

2- GADO A., EBEID B., ABDELMOHSEN A. and AXON A.: Colorectal cancer in Egypt is commoner in young people: Is this cause for alarm? Alexandria Journal of Medicine, DOI: 10.1016/j.ajme, 50 (3): 197-201, 2014.

3- Cleary S.P., Cotterchio M., Shi E., Gallinger S. and Harper P.: "Cigarette smoking, genetic variants in carcinogen-metabolizing enzymes, and colorectal cancer risk," American Journal of Epidemiology, 172 (9): 1000-1014, 2010.

4- LICHTENSTEIN P., HOLM N.V., VERKASALO P.K., ILIADOU A., KAPRIO J., KOSKENVUO M., PUKKALA E., SKYTTHE A. and HEMMINKI K.: Environmental and heritable factors in the causation of cancer analyses of cohorts of twins from Sweden, Denmark, and Finland. N. Engl. J. Med., 13; 343 (2): 78-85, 2000.

5- AL-MAGHRABI J., MUFTI S., GOMAA W., et al.: Immunoexpression of cyclin D1 in colorectal carcinomas is not correlated with survival outcome. J Microsc Ultrastruct.;3:62-67, 2015.

6- LV M., DONG W., LI L., ZHANG L., SU X., WANG L., GAO L. and ZHANG L.: Association between genetic variants in pre-miRNA and colorectal cancer risk in a Chinese population. Journal of Cancer Research and Clinical Oncology, 139 (8): 1405-1410, 2013.

7- KUSENDA B., MRAZ M., MAYER J. and POSPISILOVA S.: MicroRNA biogenesis, functionality and cancer relevance. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub; doi: 10.5507/bp.2006.029. PMID: 17426780, 150 (2): 205-15, 2006.

8- RONG G., ZHANG X., CHEN B., YANG X., WU H. and GONG W.: MicroRNA gene polymorphisms and the risk of colorectal cancer; 13: 3617-3623, Oncol Lett 2017.

9- HU X., LI L., SHANG M., et al.: Association between microRNA genetic variants and susceptibility to colorectal cancer in Chinese population. Tumour Biol., 2013.

10- ZHANG M.W., JIN M.J., YU Y.X., et al.: Associations of lifestyle-related factors, hsa-miR-149 and hsa-miR-605 gene polymorphisms with gastrointestinal cancer risk. Mol. Carcinog, 51: E21-31, 2012.

11- Liu X.X., WANG M., XU D., YANG J.H., KANG H.F., WANG X.J., LIN S., YANG P.T., LIU X.H. and DAI Z.J.: Quantitative Assessment of the Association between Genetic Variants in MicroRNAs and Colorectal Cancer Risk. Biomed Res. Int., 276410 2015. doi: 10.1155/2015/276410, PMID: 26078942; PMCID: PMC4452836, Epub May 20, 2015.

12- LIU Y., LIU R., YANG F., CHENG R., CHEN X., CUI S., GU Y., SUN W., YOU C., et al.: miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. Molecular Cancer, 16: 53, 2017.

13- XU L. and TANG W.: Associations of Polymorphisms in mir-196a2, mir-146a and mir-149 with Colorectal Cancer Risk: A Meta-Analysis. Pathol. Oncol. Res., 22 (2): 261-267, 2016.

14- KHAN N.P., PANDITH A.A., HUSSAIN M.U., YOUSUF A., KHAN M.S. and SIDDIQI M.A.: Loss of heterozygosity (LOH) of deleted in colorectalcancer (DCC) gene and predisposition to colorectal cancer significant association in colorectal cancer patients of Kashmir. J. Cancer Res. Expt. Oncol.; 3: 88-94, 2011.

15- DERKS S. and VAN ENGELAND M.: DCC (deleted in colorectal carcinoma) Atlas Genet. Cytogenet Oncol. Haematol., 14 (10): 313-316, 2010.

16- ADRIENNE V.D., JOSEPH S., ELLEN F. and MOLLY D.S.: The Drosophila netrin receptor frazzled/DCC functions as an invasive tumor suppressor. BMC Dev. Biol., 11: 41, 2011

17- DJANSUGUROVA L., ZHUNUSSOVA G., KHUSSAINOVA E., IKSAN O., AFONIN G., KAIDAROVA D. and PARKER M.I.: Association of DCC, MLH1, GSTT1, GSTM1, and TP53 gene polymorphisms with colorectal cancer in Kazakhstan. Tumor Biol., 36: 279-289, 2015.

18- BAHNASSY A.A., ZEKRI A.R., EL-HOUSSINI S., et al: Cyclin A and cyclin D1 as significant prognostic markers in colorectal cancer patients. BMC Gastroenterol., 4: 22, 2004.

19- NEVINS J.R.: E2Fa link between the Rb tumor suppressor protein and viral oncoproteins. Science, 258: 424-429, 1992.

20- BESSON A., DOWDY S.F. and ROBERTS J.M.: CDK inhibitors, cell cycle regulators and beyond. Dev. Cell, 14: 159-169, 2008.

21- ALBASRI A.M., ELKABLAWY M.A., ANSARI I.A. and ALHUJAILY A.S.: Prognostic Significance of Cyclin D1 Over-expression in Colorectal Cancer: An Experience from Madinah, Saudi Arabia. Asian Pac. J. Cancer Prev., 20 (8): 2471-2476, 2019.

22- KOURAKLIS G., THEOCHARIS S., VAMVAKAS P., VAGIANOS C., GLINAVOU A., GIAGINIS C. and SIOKA C.: Cyclin D 1 and Rb protein expression and their correlation with prognosis in patients with colon cancer. World J. Surg. Oncol., 4: 5, 2006.

23- HILSKA M., COLLAN Y.U., O LAINE V.J., et al. The significance of tumor markers for proliferation and apoptosis in predicting survival in colorectal cancer. Dis. Colon. Rectum., 48: 2197-208, 2005.

24- HOLLAND T.A., ELDER J., MCCLOUD J.M., et al.: Subcellular localisation of cyclin D1 protein in colorectal tumours is associated with p21WAF1/CIP1 expression and correlates with patient survival. Int. J. Cancer, 95: 302-6, 2001.

25- Association W.M. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA, 310 (20): 2191, 2013.

26- EDGE S.B. and COMPTON C.C.: The American Joint Committee on Cancer: the 7 th edition of the AJCC cancer staging manual and the future of TNM. Ann. Surg. Oncol., 17: 1471-4, 2010.

27- BOOM R., SOL C.J., SALIMANS M.M., JANSEN C.L., WERTHEIM-VAN DILLEN P.M. and VAN DER NOORDAA J.: Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol., 28 (3): 495-503, 1990.

28- MALIK M.A., GUPTA A., ZARGAR S.A. and MITTAL B.: Role of genetic variants of deleted in colorectal carcinoma (DCC) polymorphisms and esophageal and gastric cancers risk in Kashmir Valley and meta-analysis. Tumor Biol., 34: 3049 -57, 2013.

29- TOMA M., STAVARACHI M., CIMPONERIU D., APOSTOL P., COJOCARU M., BELUSICA L., PANDURU N., RADU I. and GAVRILA L.: P53 and DCC Polymorphisms and the risk for colorectal cancer in Romanian Patients. J Analele Universita~tii din Oradea, Fascicula. Biol., 16: 162-165, 2009.

30- HEZOVA R., KOVARIKOVA A. and BIENERTOVA-VASKU J.: Evaluation of SNPs in miR-196-a2, miR-27a and miR-146a as risk factors of colorectal cancer. World J. Gastroenterol., 18: 2827-31, 2012.

31- ALAO J.P.: The regulation of cyclin D1 degradation: Roles in cancer development and the potential for therapeutic invention. Mol. Cancer, 6: 24, 2007.

32- DU W., MA X., KONG W., LIU T., et al.: Association between rs11614913 polymorphism in miR-196a2 and colorectal cancer risk: A meta-analysis. Cancer Biomark, 13: 457-464, 2013.

33- CASTETS M., BROUTIER L., MOLIN Y., BREVET M., CHAZOT G., GADOT N., PAQUET A., MAZELIN L., JARROSSON-WUILLEME L., SCOAZEC J.Y., et al.: DCC constrains tumour progression via its dependence receptor activity. Nature, [CrossRef] [PubMed], 482: 534-537, 2011.

34- KRIMPENFORT P., SONG J.Y., PROOST N., ZEVENHOVEN J., JONKERS J. and BERNS A.: Deleted in colorectal carcinoma suppresses metastasis in p53-deficient mammary tumours. Nature, [CrossRef] [PubMed] : 541-482, 538, 2012.

35- CHANDRA V., KIM J.J., GUPTA U., MITTAL B. and RAI R.: Impact of DCC (rs714) and PSCA (rs2294008 and rs2976392) Gene Polymorphism in Modulating Cancer Risk in Asian Population. Genes, 9: 7 (2), 2016.

36- STARINSKY S., FIGER A., BEN-ASHER E., GEVA R., FLEX D, FIDDER H.H., ZIDAN J., LANCET D. and FRIEDMAN E.: Genotype phenotype correlations in Israeli colorectal cancer patients. International Journal of Cancer, 114 (1): 58-73, 2016.

37- KATHLEEN R.C., JONATHAN D.O., JONATHAN W.S., LORA H., ERIC R.F., ANTONETTE C.P., et al.: The DCC gene: Structural analysis and mutations in colorectal carcinoma. Genomics, 19: 525-31, 1994.

38- MUSTAFAA, ÇIGDEMA, FIKRETD, ÖZGÜR S, BAHADY´R M.G., AYDIN S., et al.: Clinical significance of p53, K-ras and DCC gene alterations in the stage I-II colorectal cancers. J. Gastrointest Liver Dis., 16: 11-7, 2007.

39- AKKIPRIK M., ATAIZI-ÇELIKEL Ç, DÜS ̧ÜNCELI F., SÖNMEZ Ö., GÜLLÜOGLU B.M., SAV A., et al.: Clinical significance of p53 K-ras and DCC gene alterations in the stage I-II colorectal cancers. J. Gastrointest Liver Dis., 16: 11-7, 2007.

40- RASOOL S., RASOOL V., NAQVI T., GANAI B.A. and SHAH B.A.: Genetic unraveling of colorectal cancer: Tumour Biol., 35: 5067-82, 2014.

41- SHI L., ZHANG C., ZHAO D., LIU K., LI T. and TIAN H.: Mir-196a-2 C>T polymorphism as a susceptibility factor for colorectal cancer. Int. J. Clin. Exp., 8 (2): 2600-2606, 2015.

42- WANG J., WANG Q., LIU H., SHAO N., TAN B., ZHANG G., WANG K., JIA Y., MA W., WANG N. and CHENG Y.: The association of miR-146a rs2910164 and miR-196a2 rs11614913 polymorphisms with cancer risk: A meta-analysis of 32 studies. Mutagenesis, 27: 779-88, 2012.

43- PARLAYAN C., IKEDA S., SATO N., SAWABE M., MURAMATSU M. and ARAI T.: Association Analysis of Single Nucleotide Polymorphisms in miR-146a and miR-196a2 on the Prevalence of Cancer in Elderly Japanese: A Case-Control Study. Asian Pacific Journal of Cancer Prevention, 15 (2): 234-241, 2014.

44- CHEN H., SUN L.Y., CHEN L.L., ZHENG H.Q. and ZHANG Q.F.: A variant in microRNA- 1 96a2 is not associated with susceptibility to and progression of colorectal cancer in Chinese. Intern. Med. J., 42: e115-9, 2011.

45- PAN X.M., XIAO X., QIN H.J., ZHANG Z., LI Z.H., GAO LB. and JIA J.: MicroRNA variants and colorectal cancer risk: A meta-analysis. Genetics and Molecular Research, 15 (3): gmr.15038478, 2016.

46- ZHU L., CHU H., GU D., MA L., SHI D., ZHONG D., TONG N., ZHANG Z. and WANG M.: A functional polymorphism in miRNA-196a2 is associated with colorectal cancer risk in a Chinese population. DNA Cell Biol., 31: 350-4, 2012.

47- ZHAN J.F., CHEN L.H., CHEN Z.X., YUAN Y.W., et al.: A functional variant in microRNA-196a2 is associated with susceptibility of colorectal cancer in a Chinese population. Arch. Med. Res., 42: 144-148, 2011.

48- JING L., SU L. and RING B.Z.: Ethnic Background and Genetic Variation in the Evaluation of Cancer Risk: A Systematic Review. PLoS ONE, 9 (6): e97522, 2014.

49- MOHAMED S.Y., HAFEZ A., ELWAN A., ABDELHAMID M.I., AHMED N.H., ABDELMAKSOUD B.A. and ABDELBARY A.M. Prognostic Value of IMP3 and Cyclin D1 Expression in Patients with Colorectal Cancer. Middle East Journal of Cancer, doi:10.30476/mejc.2021. 85085.1258, 2021.

50- OGINO S., NOSHO K., IRAHARA N., KURE, SHIMA K., BABA Y., TOYODA S., CHEN L., GIOVANNUCCI E.L., MEYERHARDT J.A. and FUCHS C.S.: A Cohort Study of Cyclin D1Expression and Prognosis in 602 Colon Cancer Cases. Clin. Cancer Res., 4431; 15 (13), 2009.

51- SCHERNHAMMER E.S., TRANAH G.J., GIOVANNUCCI E., CHAN A.T., MA J., COLDITZ G.A., HUNTER D.J., WILLETT WC. and FUCHS C.S.: Cyclin D1 A870G polymorphism and the risk of colorectal cancer and adenoma British Journal of Cancer, 94: 928-934, 2006.

52- FORONES N.M., DE LIMA J.M., DE SOUZA LG., DA. and SILVA I.: Cyclin D1 A870G Polymorphism in Brazilian Colorectal Cancer Patients. J. Gastrointest Canc., 39: 118-123, 2008.

دراسة العلاقة بين مستوى مادة سيكلين د 1 وتعدد الأشكال الجينيه لجين حمض الريبونيوكليك الصغير وجين الممحى فى سرطان القولون (دى سى سى جين) فى مرضى سرطان القو لون والمستقيم (a196مير)

المقدمة : يحتل مرض سرطان القولون والمستقيم المرتبة الرابعة لأكثر الأورام شيوعاً بين المصريين، حيث تبلغ نسبة الإصابة بهذا المرض ٤.٣٪ من إجمالى عدد حالات قسم الأورام بمستشفى قصر العينى - جامعة القاهرة.

هدف الدراسة : الهدف من هذا البحث هو دراسة العلاقة بين الأشكال الجينية المتعددة لجين حمض الريبونيوكليك وجين الممحى فى سرطان القولون (دى سى سى) مع مستوى سيكلين د ١ فى الدم فى مرضى (a196 مير) الصغير سرطان القولون والمستقيم مقابلاً للأصحاء، وذلك لدرسة مدى ارتباط هذه الجينات الوراثية بخطر حدوث سرطان القو لون والمستقيم. وكذلك يهدف البحث إلى ربط هذه الأنماط الجينية مع الورم المستئصل جراحياً، من حيث مكان الورم، ودرجته، وتصنيفه تحت المجهر، والانبعاثات البعيدة. وكذلك ربط مستوى سيكلين د ١ فى الدم مع حدوث سرطان القولون والمستقيم.

طرق البحث : أجريت هذه الدراسة على مائة وستين شخصاً، المجموعة الأولى مائة مريض يعانى من مرض سرطان القولون. والمجموعة الثانية تتكون من ستين شخصاً من الأصحاء ثبت خلوهم من المرض. تمت دراسة تعدد الأشكال الجينية من خلال تحليل جين الممحى فى سرطان القولون باستخدام تفاعل البلمرة المتسلسل فى طريقة تقييد طول قطعة تعدد الأشكال، بعمل تحليل تفاعل البلمرة المتسلسل وكذلك تم (a196) وتعدد الأشكال الجينية للجين حمض الريبونيوكليك الصغير (مير٢) قياس مستوى بروتين سيكلين د فى الدم بعمل تحليل الا ليزا

النتائج : أسفرت الدراسة عن وجود علاقة وثيقة بين أنماط جين الممحى فى سرطان القولون (دى سى سى جين ا) وسرطان القولون والمستقيم. كانت نسبة الجين تساوى نسبة ٧٦٪ فى المرضى و ٥٥٪ فى الأصحاء، كذلك وجدت زيادة نسبة الانبعاثات البعيدة بين المرضى حاملى النمط الجينى مما يرجح أم هذه الطفرة مرتبطة بزيادة نسبة حدوث الأورام المتشعبة. كما وجدت علاقة بين مستوى بروتين سيكلين د١ فى الدم وزيادة نسبة حدوث سرطان القولون والمستقيم.

الخلاصة : نستخلص من هذا البحث أن كلا من جين الممحى فى سرطان القو لون (دى سى سى نمط جى جى) ومستوى بروتين سيكلين د ١ فى المصل مرتبطان ارتباطاً وثيقاً بارتفا ع خطر الإصابة بسرطان القولون والمستقيم فى المصريين، وينذر كذلك بخطر الإصابة بالأورام المتقد مة، ونقترح أن يتم اسغلا له فى التشخيص المبكر لسرطان القولون والمستقيم وكمؤشر على خطورة الورم.