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Expression and Polymorphism of Long Non-Coding RNAs in Colorectal Cancer

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ABSTRACT

Aim of work: to conduct a clinical biochemical study that aids in investigation of some non-coding RNA expressions and polymorphisms (including long non-coding RNAs and miRNAs) namely, PVT-1 and miR-186 in an attempt to provide new noninvasive diagnostic biomarkers for colorectal cancer(CRC)of the Egyptian patients.

Patients and methods: Eighty CRC studies and thirty healthy controls were included. Laboratory and pathological investigations were assessed. Serum miR-186 and long non-coding PVT-1was measured as well as genotype for PVT-1rs13255292.

Results: The CRC patients had a mean age of 50.91±12.0. The mean serum miR-186 level in CRC patients (1.38±0.21) was significantly higher than in the control (0.93±0.1) (p=0.0001). The PVT level in CRC patients was (5.91±0.25) significantly higher than control (1.1±0.2) (p=0.001). The PVT-1 rs13255292 genotype CT was significantly higher compared to the control group. The T allele was highly significant with p-value 0.008 in CRC patient group as regards to the control.

Conclusion:miR-186, long non-coding PVT-1 as well as PVT-1the T allele may be considered genetic markers for CRC susceptibility.

KeywordsmiRNAs; lncRNA; CRC; SNPs

1. Introduction

CRC is considered as one of the most widely spread cancer diseases that develop as a consequence of both genetic and environmental risk factors. There are various habits and environmental factors that are frequently associated with CRC risk such as diet, smoking and drinking habit (1, 2). It is also considered one of the best- characterized cancers and a leading cause of cancer death worldwide (3)**.**

A large number of evidences has demonstrated that non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have functional roles in both the physiological and pathological processes by regulating the expression of their target genes. These molecules are engaged in the pathobiology of neoplastic diseases and are targets for the diagnosis, prognosis and therapy of a variety of cancers, including CRC (4).

MicroRNAs (miRNAs; miRs) are the small, single-stranded non-coding RNAs that are 18–24 nucleotides (nt) in length and affect the gene expression of their target genes via posttranscriptional regulation (5). Currently, thousands of miRNA loci have been identified, with the increased number of studies in humans, some miRNAs have been found to be encoded from the same loci on chromosomes but have different target mRNAs, and others have synergistic effects on the same mRNA (6).

Similar to genes, miRNAs can be divided into three groups: tumor suppressor miRNAs, oncogenic miRNAs, and metastatic miRNAs (6). miRNAs can regulate not only several pathophysiologic events but also cell proliferation and differentiation (7).

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miR-186 has recently drawn considerable attention as among the abundant cancer-associated miRNAs, which may act as either an onco-miR or a tumor suppressor. miR-186 plays an important role in early detection of cancer were its effect on multiple tumor biological behaviors, including cell proliferation, apoptosis, migration, invasion, cell cycle, intracellular metabolism, as well as the angiogenesis and lymphangiogenesis of tumors is very high(8).

Long non-coding RNA (LncRNA) is one of these and consists of >200 nucleotides. It is abnormally expressed in many human diseases and has an influence on both the pathogenesis and maintenance of disease status (9). Notably, the tumorigenicity and tumor suppression of lncRNA plays an important role in many types of tumors, including CRC. Additionally, the close association between dysregulated lncRNAs and the diagnosis and prognosis of CRC patients indicated their potential capacity as novel biomarkers or therapeutic targets (10).

Single-nucleotide polymorphisms (SNPs), the most frequent form of genetic variation as recently, 1000 Genomes Project has summed up more than 38 million SNPs (nearly a half was previously unknown) in 14 different populations worldwide (11). SNPs in miRNA genes and in different categories of non-coding RNAs including lncRNAs may have effects on CRC risk, prognosis and treatment response. In addition, miRNAs alone are thought to regulate expression of more than 1/3 of human protein- coding genes. Thus, in turn, each miRNA may potentially regulate hundreds of potential targets in the human genome. The identification of allele specific miRNA:mRNA interactions may help us to understand the role of many SNPs for which functionality is still unknown (12).

PVT-1 is a novel lncRNA, whose function remains totally unclear. So, for the first time we are going to assess serum PVT-1 gene polymorphism and miR-186 expression level in CRC. As identification of the correlation between them would be useful in understanding the disease biology and predicting important diagnostic and prognostic parameters in CRC.

2. Subjects and methods

Eighty CRC patients fulfilling the rules of the Declaration of Helsinki 1975.Ethical committee approval was taken from Faculty of Medicine, Cairo University as well as a written consent from each subject before the start of the study as well.

Thirty healthy control studies were included as well. Full history taking, clinical examinations, as well as laboratory investigations were performed on all subjects. Detecting the site and the tumor type were included as well with a full complete colonoscopy picture to all patients.

From all participants, three mL peripheral blood sample was withdrawn from each subject by venipuncture in dry sterile vacutainer tube which was taken in plain tube for serum separation that was used in detecting all serological markers for CRC, and serum was stored at –80°C for RNAs extraction including noncoding RNA and detection of fold change of the 2 non coding genes (microRNAs-186 and PVT-1) using real time PCR. In addition, two mL whole blood was taken on EDTA for DNA extraction then genotyping of the studied SNP [rs13255292(C/T)] using real time PCR.

2.1 Molecular biology techniques:

- miR-186 expression

For the expression of miR-186, RNA was extracted from serum using (Qiagen, Valencia, CA, USA). After that, RNA samples were subjected to RNA quantitation and purity assessment using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA). Followed by the reverse transcription (RT) which was carried out on total RNA in a final volume of 20 uL RT reactions using the miScript II RT kit (Qiagen, Valencia, CA, USA).Quantitative Real-time PCR (qPCR) for Detection of Mature miRNA took place where the reaction mix was prepared according to the Quanti-Fast SYBR Green PCR Kit delivers fast and specific quantification of cDNA target by real-time PCR using SYBR Green I detection. Regarding the calculation of results, melting curves analyses were performed to validate the specific generation of the expected PCR product. As there is no known control miRNA in serum, SNORD 68 was used as an endogenous control. Also GAPDH was used as endogenous control for long noncoding PVT-1. The expression level of miR-186 and PVT were calculated relative to the control samples (used as the calibrator sample) using the formula ($2^{-\Delta\Delta CT}$) and were expressed as fold-change.

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- Detection of genotyping [PVT-1 rs13255292(C/T)]

DNA was extracted from whole blood using Qia-amplification DNA extraction kit (Qiagen, USA). After that, DNA samples were subjected to DNA quantitation and purity assessment using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA).Genotyping of PVT related SNP (rs13255292(C/T)) was performed using real-time polymerase chain reaction with TaqMan allelic discrimination assay. A predesigned primer/probe sets for the genotypes were used. Probes were synthesized with reporter dye FAM or VIC covalently linked at the 5′ end and a quencher dye MGB linked to the 3′ end of the probe (Applied Biosystems, USA).

2.2 Statistical Analysis:

Data Analysis was performed using statistical package of social science (SPSS 17.0) on windows 8.1. For quantitative parametric data, Independent student t-test was used to compare measure of 2-independent groups as well as One-way ANOVA test was used for comparing more than 2-independent groups with Benferroni Post-Hoc to test significance at p-value <0.05. While for quantitative non-parametric data, Kruskalwallis test and Mann-whitney test were used to compare more than 2-independent groups. For measuring the correlation between qualitative data, Bivariate Pearson correlation test to find out the association between different groups with a two-tailed to test the significance. Sensitivity and specificity test were generated for testing a new test with ROC Curve (Receiver Operating Character). P-value<0.05 was considered as a cutoff value for significance.

3. Results

There were 80 CRC subjects with a mean age of (50.91±12.0 years). The study included 30 aged healthy controls (46.97±9.65 years). Results showed that there was no statistically significant between the two groups with respect to age and gender with p-value 0.60 and 0.78 respectively.

According to the laboratory data and the routine investigations, there was no statistical significance between the two groups as regards toplatelets, ALT, AST, Bilirubin or Albumin with p-value (0.10, 0.78, 0.87, 0.49 and 0.6 respectively). There was a significant between hemoglobin within the two groups (Control and CRC patients) with mean±SD of **(**12.50±1.1 and 10.98±2.94) respectively and p-value 0.05.

Table(1): Demographic and laboratory data of studied groups

Variables	**CRC (n=80)**	**Control (n=30)**	**p-value**
Age (years)	50.91±12.0	46.97±9.65	0.60 [a]
Gender			0.78 [b]
Female	26 (32.5%)	14(46.7%)	
Male	54 (67.5%)	16(53.3%)	
Hg(12-16 g/dL)	10.98±2.94	12.50±1.1	**0.05[a]**
Platelets(150-450 10^3/cmm)	185.55±131.83	177±100.0	0.105[c]
ALT(0.0-42 IU/L)	21.925±10.07	22.2±9.07	0.779 [c]
AST(0.0-42 IU/L)	24.125±10.69	25.67±8.1	0.872 [c]
Bilirubin(0.0-1.0 mg/dL)	0.74±0.34	0.88±0.15	0.494 [c]
Albumin(3.5-5.5 g/dL)	4.43±0.94	4.73±0.4	0.6 [c]

Age is shown as mean ±SD, and gender is presented by n (%).

P values in bold are statistically significant (P < 0.05).

a, One-way ANOVA; b, Chi-squared test;. c, Independent t-test

Hg, hemoglobin; AST, aspartate transaminase; ALT, alanine transaminase; ANOVA, analysis of variance; BIL, bilirubin; SD, standard deviation.

The clinical and pathological features for CRC group are shown in table (2).

Table(2): Clinical and pathological features of studied groups

Parameters	**N(%)**

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Symptoms of presentation	
Abdominal pain	49(61.25%)
Constipation	55(68.75%)
Loss of weight	31(38.75%)
Bleeding per rectum	18(2.25%)
Type of Tumor	
Adenocarcinoma	66(82. 5%)
Mucoid	14(17.5%)
Site	
Ceacum+Ascending	13(16.25%)
Transverse+Flexures	18(22.5%)
Rectum+Sigmoid	49(61.25%)
Colonoscopy Picture	
Colonoscopy Mass	41(51.25%)
Colonoscopy Ulcer	22(27.5%)
Colonoscopy Hyperemia	12(15%)
CT Picture	
Mass Lesion	29(36.25%)
Wall Thickening	32(40%)
Regional LNs	33(41.25%)
Hepatomegaly	9(11.25%)
Liver Mets	9(11.25%)
Tumor Grades	
Adenocarcinoma	
Grade I	33 (41.25%)
Grade II	22 (27.5%)
Grade III	11(13.75%)
Mucoid	
Grade II	14(17.5%)

Ten cases (12.5%) had both Ulcer and mass in their colonoscopy picture at the same time. Fifteen cases (18.75%) had mass lesion and regional LNs lymph node in the CT analysis.

Serum biomarkers level in CRC patients and control group

miR-186 and LncPVT-1 gene expression examined in serum regarding CRC group compared to healthy controls. Both expressions were highly statistically significant with p-value 0.0001 each(Table 3).

Table (3): Serum biomarkers level in CRC patients and control groups

Biomarkers	**CRC**	**Control**	**p-value**

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miR-186	1.38±0.21	0.93±0.1	**0.0001a**
LncPVT-1	5.91±0.25	1.1±0.2	**0.0001a**

P values in bold are statistically significant (<0.05).
a One-way ANOVA

Relationship between serum biomarkers miR-186, lncPVT-1 and different characteristics in colorectal cancer group

As regards the site of the cancer, the patients were classified into three different groups: (a) Ceacum + Ascending; (b) Transverse + Flexures and (c) Rectum + Sigmoid.

Regarding miR-186 and the pathological data, results showed that there is a statistically significant between miR-186 and the site of cancer between groups a, b with p-value 0.05. There was also a statistically significant between group a,c with p-value 0.016 while there was no statistically significant between groups b, c with p-value 0.8.

For lncPVT-1, there was also a statistically significant as regards to the pathological data, where between group a, b with p-value 0.016, there was also a statistically significant between group a, c with p-value 0.042 while there was no statistically significant between groups b,c with p-value 0.08.

Also, the comparisons of miR-186 fold change levels and colonoscopy analysis among CRC patients as well as PVT-1 levels where taken place. Colonoscopy picture was classified into mass, ulcer and hyperemia. For both miR-186 and PVT-1, there was no statistical significance between patients having mass or ulcer& hyperemia and patients who have not where p-value>0.05.

Regarding miR-186 and computed tomography scan (CT Picture), there were high statistically significant between patients with and without (Wall Thickening, Regional LNs and Liver Mets) with p-values (0.007, 0.016 and 0.05). Also for miR-186, there were no statistically significant between patients with Mass Lesion and Hepatomegaly where p-value>0.05.

For PVT-1 and CT picture, there were no statistically significant between any of the CT scan and the PVT-1 expression.

There was no statistically significant between miR-186 and tumor types (Adenocarcinoma and Mucoid) with p-value 0.702. While there is statistical significance with LncPVT-1 with p-value 0.05 [Table (4)].

Table (4): Relationship between serum biomarkers miR-186, long non coding PVT-1 and different characteristics in colorectal cancer group

Parameters			**miR-186 Mean±SE**	**p-value**	**PVT-1 Mean±SE**	**p-value**
Site of anatomy	Ceacum + Ascending		0.717±0.30	**0.05a**	1.31±0.39	**0.016a**
	Transverse + Flexures		1.57±0.33	**0.016b**	5.57±0.54	**0.042b**
	Rectum + Sigmoid		1.62±0.29	0.8	5.92±0.33	0.08
Colonoscopy Picture	Mass	Yes	1.24±0.22	0.181	6.22±0.33	0.102
		No	1.84±0.48		6.51±0.36	
	Ulcer	Yes	0.90±0.27	0.062	5.80±0.65	0.238
		No	1.68±0.30		6.52±0.29	
	Hyperemia	Yes	1.55±0.34	0.18	5. 0±0.5	

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		No	1.8±0.20		6.2±0.9	0.31
CT Picture	Mass Lesion	Yes	1.43±0.32	0.887	5.65±0.48	0.230
		No	1.52±0.33		6.75±0.31	
	Wall thickening	Yes	0.88±0.15	**0.007**	6.68±0.41	0.587
		No	1.94±0.38		6.08±0.36	
	Regional LNs	Yes	0.85±0.20	**0.016**	5.99±0.46	0.303
		No	2.00±0.37		6.62±0.32	
	Hepatomegaly	Yes	1.24±0.001	0.48	6.89±0.001	0.79
		No	1.51±0.24		6.33±0.28	
	Liver Mets	Yes	0.71±0.32	**0.05**	5.47±0.64	0.784
		No	1.58±0.26		6.44±0.29	
Tumor Type	Adenocarcinoma		1.52±0.23	0.702	5.72±0.28	**0.05**
	Mucoid		1.32±0.45		6.75±0.43	

P values in bold are statistically significant (<0.05).
a,Ceacum+Ascending&Transverse+Flexures;b,Ceacum+Ascending&Rectum+Sigmoid; c, Transverse+Flexures&Rectum+Sigmoid

Correlations of miR-186 levels and PVT-1fold change with demographic and laboratory parameters in CRC patients

Regarding the correlation study, there was no correlation between the demographic features age, sex or obesity. There was also no correlation either between laboratory data with miR-186 or PVT-1 (ALT; AST; BIL; ALB). While there was a positive correlation betweenlncPVT-1 and platelets with r=0.38 and p value=0.0004 [Table (5)].

Table(5): Correlations of miR-186 fold change levels with demographic and laboratory parameters in CRC patients

Parameters	**miR-186 (r, p-value)**	**PVT-1 (r, p-value)**
Age	-0.048 (0.66)	0.0001 (0.99)
Sex	-0.027 (0.814)	0.024 (0.832)
Obesity	-0.116 (0.394)	-0.045 (0.741)
PLT	-0.004 (0.96)	0.38 (**0.0004)**
ALT	-0.141 (0.212)	0.070 (0.538)
AST	-0.128 (0.259)	0.063 (0.579)
BIL	0.094 (0.405)	-0.153 (0.176)
ALB	0.005 (0.966)	0.195 (0.083)

r, Pearson correlation; PLT, platelets; AST, aspartate transaminase; ALT, alanine transaminase; BIL, bilirubin; ALB; Albumin. P values in bold are statistically significant (<0.05).

ROC curve analysis for serum miR-186 and lncPVT-1

The ROC curve analysis of serum miR-186 and PVT-1 in colorectal cancer patients is determined in table 6. Diagnostic performance of different genes expression as markers of CRC group at different cutoff points using ROC curve. The calculated sensitivity, specificity, and diagnostic accuracies for studied parameters to discriminate CRC patients from healthy control (Table 6). For discrimination of CRC group versus control group, regarding miR-186, the AUC = 0.525 with sensitivity of 47% and specificity of 100%, while for LncPVT-1, the AUC was of 0.975 with sensitivity of 73.75% and specificity of 100% (Figure 1).

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Table (6): Diagnostic and prognostic performances of miR-186 & LncPVT-1

Serum Biomarkers	AUC	Cut-off value	Sensitivity	Specificity	95% C.I	Accuracy	p-value
miR-186	0.525	1.36	47.5%	100%	0.408 to 0.630	73.7%	**0.0001**
PVT-1	0.975	4.55	73.75%	100%	0.940 to 1.000	86.8%	**0.001**

P values in bold are statistically significant (P < 0.05).
AUC, area under curve.

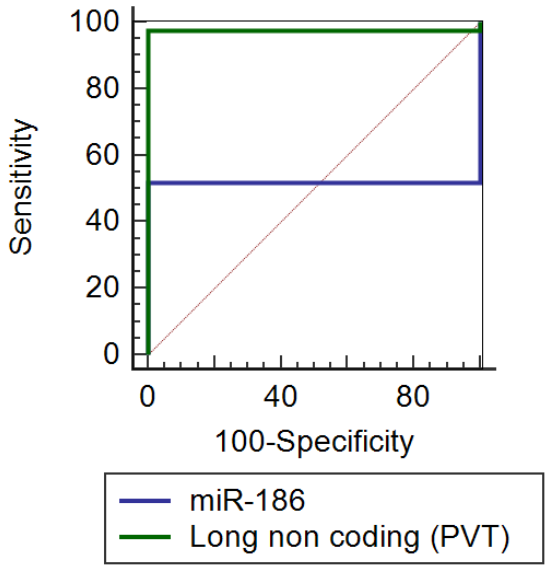

Figure 1: ROC Curve for miR-186 and PVT-1 in CRC patient group

Genotypes and Allelic distribution of PVT-1 rs13255292 C/T in Colorectal cancer and control group

The genotypes and allele distributions of PVT-1 rs13255292 C/T in cases (Colorectal cancer and control groups) are shown in table (7).The rs13255292 genotype distributions in controls were in accordance with Hardy-Weinberg equilibrium (p=0.380). Our results revealed that genotype CC was lower in CRC group with 41.25% compared to the control group with 43.4%. While CT genotype was 46.25% and 23.3% in CRC groups and control group respectively. TT genotype was higher in control group compared to CRC group with percentage of 33.3 and 12.5 respectively.

On comparing the studied parameters according to the PVT-1 / rs13255292 genotype, there was no significant difference with CC genotype as regard to the control group. While comparing the studied parameters according to PVT/ rs13255292 genotype, in those with CT genotype and TT, there were significant with respect to the control group with p-value 0.010 and 0.001 respectively.

Table(7): Genotypes and Allelic distribution of PVT-1 rs13255292 C/T in Colorectal cancer and control group

PVT-1 rs (rs13255292)	CRC	Control	p-value
Genotypes			
CC	33(41.25%)	13(43.4%)	0.8

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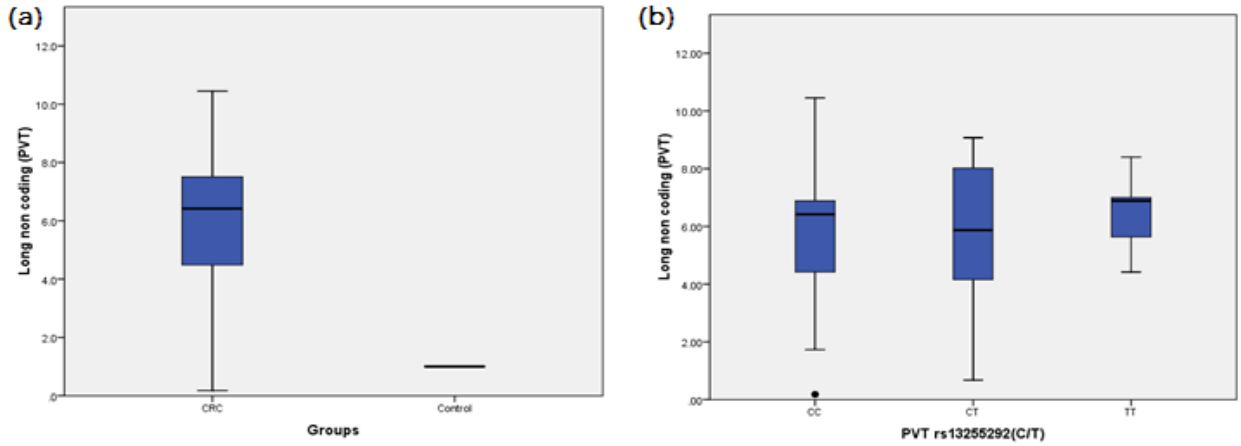
CT	37(46.25%)	7(23.3%)	**0.010**
TT	10(12.5%)	10(33.3%)	**0.001**
Alleles			
C	103	33	**0.001**
T	57	27	**0.008**

Hardy-Weinberg equilibrium; X2 = 0.77, P = 0.380. P values in bold are statistically significant (<0.05).

Increased serum PVT-1 was observed in the colorectal cancer group when compared with control group with p-value 0.0001. There is also increases genotype expression CT and TT in CRC groups with p-values (0.010 and 0.001) [Figure(2)].



Figure(2): Relative serum expression level of lncRNA(PVT-1) and genotypes

Homogeneity analysis of rs13255292 by age and gender in colorectal cancer group

We further stratified the relation between the age and gender across the patients, results reported that there were no high risk for all genotypes PVT-1 rs13255292 neither for age nor gender with p-value>0.05.

Table(8): Homogeneity analysis of rs13255292 by age and gender in colorectal cancer group

Parameter		**Genotypes in CRC patients (*n* = 80) Mean ± SE**			
		PVT-1 rs13255292			
		CC	**CT**	**TT**	**p-value**
Age		51.61±2.24	50.68±1.96	51.11±2.94	0.52
Gender		**Count(%)**			0.267
	F	11(13.75%)	10(12.5%)	5(6.25%)	
	M	23(27.5%)	27(33.75%)	5(6.25%)	

Relationship between genotypes of PVT-1 **rs13255292** *and different characteristics in colorectal cancer group*

Regarding the site of cancer, TT genotype percentage was higher in patients with (Rectum or Sigmoid) followed by CC genotype in the same site with p-value 0.003.As for tumor types, a significantly high percentage of patients who have TT genotype are mucoid type, followed by CC and CT genotypeswith p-value 0.001(Table 9).

Table(9): Relationship between genotypes of PVT-1 rs13255292 and different characteristics in CRC group

Parameter	**Genotypes in CRC patients (*n* = 80) Mean ± SE**			
	PVT-1 rs13255292			
	CC	**CT**	**TT**	**Crude OR (95% CI), P[a]**

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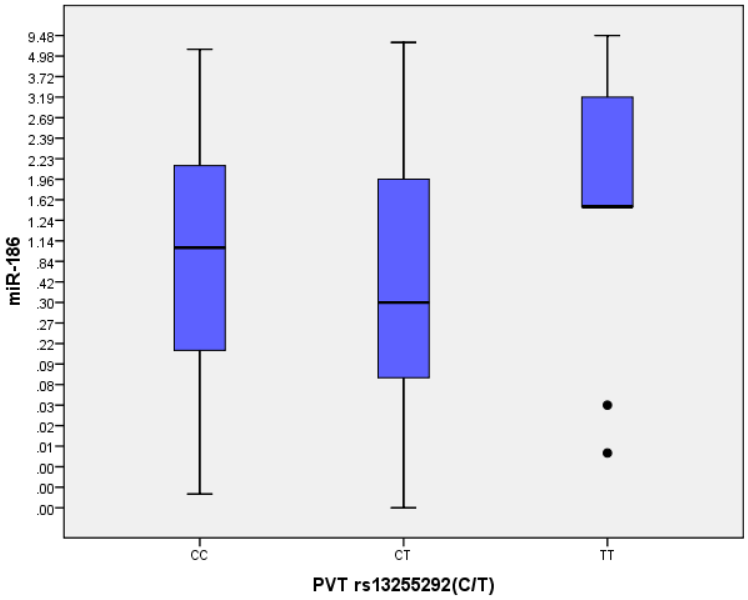
Site				1.714 (0.865–3.398); 0.003
Ceacum + Ascending	5(6.25%)	4(5%)	3(3.75%)	
Transverse + Flexures	3(3.75%)	14(17.5%)	1(1.25%)	
Rectum+Sigmoid	72(90%)	62(77.5%)	76(95%)	
Tumor Types				0.292 (0.138–0.616); 0.001
Adenocarcinoma	26(32.5%)	37(46.25%)	9(11.25%)	
Mucoid	54(67.5%)	43(53.75%)	71(88.75%)	

P values in bold are statistically significant (<0.05).
aCrude P value done by Univariate logistic regression.

Association of genotypes of PVT-1 rs13255292 & miR-186 in CRC group

Results showed that there was an association between TT genotype and miR-186 followed by CC genotype. miR-186 is up-regulating in TT genotype with 1.5 as expression level and p-value0.001.



Figure(3): Association of genotypes of PVT-1 rs13255292 miR-186 in colorectal cancer group.

4. Discussion

As general, cancer is a highly complex disease which is mediated by the interaction of various signaling pathways from tumor to metastasis. Due to unfavorable modern dietary habits, junk food as well as increase in risk factors such as low physical exercise, obesity, alcohol and smoking, the rise of colorectal cancer took place in most of the developed countries (13, 14).

Although the death rates for colorectal cancer had been improved globally as a results of the medical improvements and medications, but it had been reported that about 13 million people as estimated to die in the coming 15 years of cancer as general (15).

Colonoscopy is one of the main factors for CRC screening, but it is an invasive method. So, in our study we proposed new blood-based non-invasive genomic markers based on PCR techniques which appear both reproducible and cost-effective (16).

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The early detection of colorectal cancer is important for controlling the progression of the disease. Various studies verified that microRNAs play important roles as biomarkers for CRC that provide a new, less-invasive technique to screen for CRC and determine prognosis as well (17-24).

Studies showed that lncRNAs as well have an effective role in early detection and treatment of various cancer diseases including colorectal cancer (25).

This case study was conducted on one-hundred and ten cases that are classified into eighty CRC patients and thirty healthy controls. We measured the expression level of miR-186 and lncPVT-1 as well as PVT-1 genotype rs13255292. From our research we realized that miR-186 expression play an important role in different types of cancers including colorectal cancer, ovarian, as well as hepatic diseases (26).

Results revealed a significant up-regulation of miR-186 serum expression in CRC patients when compared with healthy control (p-value<0.05). These results are in agreements with recent study reported a significant high expression of miR-186-5p in cancer tissues ($p< 0.001$) and cell lines ($p<0.05$) when compared to control study (27). Different studies reported various results regarding the expression levels of miR-186 both up regulation and down regulation for cancer diseases. As for CRC, our results agreed with the study of F. Islam in 2017 where miR-186 expression was highly over expressed in patients with colon cancer (Islam). While there were other contradictions that the level of miR-186 was down regulated in CRC patient groups.(26,28,29)

Recent studies have confirmed the potential use of tumor-specific miRNAs as diagnostic or prognostic markers for cancer in the body fluids (30). This finding is consistent with (31, 32) who reported that, miR-186 itself is regulated by several factors, in addition to the potential significance of miR-186 in the diagnosis, prognosis and treatment of human cancer.

Both PVT-1 and miR-186 play an important role in various diseases, for instance in gliomamicro vascular endothelial cells the negative regulation of lncRNA PVT-1 on miR-186 was also observed (33).

LncPVT-1 is highly spread to be one of the parameters that is effective in many cancers. In gastric cancer, lncPVT-1 expression levels are reported to highly increase compared to the healthy cases (34).

As for our knowledge, lncPVT-1 is considered as a new parameter for detecting colon cancer and showing the correlation with tumor types or even with clinical pathological parameters. But PVT-1 reported to be over expression in CRC. As for LncPVT-1 expression level, our results showed that there was a highly significant increased PVT-1 levels in CRC group when compared with healthy control. These results are consistent with previous data reported in 2020 (35). There were also disagreements to our results related to the expression on LncPVT-1 with other cancer diseases like prostate cancer cells where proliferation and migration were significantly inhibited and highly decreased, compared to the controls (27).

As for the laboratory parameters and its relationship between the serum biomarkers, our results showed that patients with CRC have low levels of hemoglobin than normal ones, which agree the results of Shaker O., et al in 2019. Previous studies reported that anemia is a common complication of cancer where cancer patients have a significant lower Hg level than control group (36, 37).

For the clinical and pathological parameters, our results revealed that both miR-186 and PVT-1 expression levels were higher in patients with colon cancer in either Ceacum + Ascending or Transverse + Flexures than in rectum or sigmoid.

There results faced some disagreements where in 2017 for colorectal cancers, miR-186-5p was over expressed in distal colorectum than other sites of colon (26).

For the CT scan, our results reported that patients who had no wall thickening, regional LNs nor liver metastasis had the expression level of miR-186 to be higher. That also contradicted the results of F. Islam in 2017 where patients with metastasis had shown high expression of miR-186-5p than patients who don't have (26).

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As for lncPVT1 and its relation with tumor type, results showed that patients with tumor typemucoid are higher in expression level than adenocarcinoma. Results showed as well that there is a positive correlation between lncPVT1 level and palettes counts.

We assessed LncPVT-1 SNP rs13255292for CRC in Egyptian patients and as for our knowledge; it is a new pathway in colon cancer. We found that CTheterozygous genotype percentage was higher in patients than CC genotype. We also reported that the T allele was high significant in CRC patients compared to C allele in healthy group.Rs13255292 for PVT-1 was used in determining patients with glioma, where in 2017; authors reported that CT genotypes were also higher in glioma patients (38).

We further investigated the correlation between clinical and pathological parameters (site and tumor-related data) andLncPVT-1 rs13255292 genotype for CRC patients. Results revealed that CT genotype was highly significant colon site Rectum and Sigmoid than in transverse and flexures. TT genotype was high in rectum and sigmoid colons. There are considered as good findings that the polymorphism mutation with T allele can be considered as an indicator for detecting colon cancer in Egyptian patients.

As regards to tumor-types, CT and TTgenotype were reported to be high in patients categorized as having mucoid than adenocarcinoma tumor type.

Regarding PVT-1 polymorphisms, different SNPs other than what we considered in our study were used for early detection of other type of cancer like lung adenocarcinoma. In 2020, there was a study that reported different PVT-1 SNPs to be significant with CT genotypes and lung adenocarcinoma in a Chinese northeast population having T allele as a high significant factor (39).

As regarding to the association of genotypes of PVT-1 rs13255292 and miR-186 in colorectal cancer group, we reported that the mean expression level of miR-186 in PVT-1 TT genotype was about 1.51 which was high and significant. The findings in this study proved that both serum biomarkers miR-186 and lncPVT-1 could be used as clinical marker for early detection of CRC in patients as for other cancer diseases reported in 2017 (32, 38).

5. Conclusions

In conclusion, both rs13255292 and high serum PVT-1 confer increased risk for colorectal cancer development; also, a significant difference in serum miR-186 expression level was found to be associated with different pathological and laboratory findings. Our results suggested that the interaction of miR-186 with LncPVT-1 regulating gene expression and strongly prompt us to use these two parameters as noninvasive prognostic biomarkers and new therapeutic targets for CRC patients.

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