Research Article

Investigating The variations in The expression level of CEA and TIMP-1 markers in Human Colorectal Carcinoma in Egyptian Patient

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Abstract
Background/Aims: In this current study, Our aim is studying verification and confirmation of the use of two markers of colorectal cancer in Egyptian patients to discover the disease and diagnose it early, because the deaths associated with it are constantly increasing and very worrying, and there are previous studies, which some have reported that confirmed the high level of these markers in Blood plasma, when colon cancer occurs and some other studies have not been confirmed, and we did this study to verify their association with the disease in the presence of an increase in their level in blood plasma in Egyptian patients with the disease than healthy people or not related and these two were chosen CEA and TIMP-1 because they have found them linked to many different types of cancers and we want to work on using them in the early diagnosis of colorectal cancer to avoid death from it and improve the diagnosis in the initial stages of its occurrence so that we can quickly cure it and get rid of it forever and improve the level of survival rate when an injury occurs in the Egyptian society and It has been proven from this study that both the CEA and TIMP-1 concentration is present in the blood plasma with an increase in patients than in healthy humans. **Materials and Methods:** Blood samples were collected from eighty-four Egyptian patients. They were categorized into 64 patients with CRC: males 45.5% and females 54.5% with age range from 23 to 73 years (Mean ± SD: 45.8 ± 12.4 years); and 20 healthy controls: males 55% and females 45% with age range from 25 to 67 years (Mean ± SD: 43.7 ± 10.8 years). **Results:** A clear increase in the concentration of markers (CEA, TIMP-1) included in the study in blood plasma in patients more than healthy with a wide and clear difference, which indicates the correlation of the increase with the presence of the disease .**Conclusion:** It has been verified and confirmed in this study that both markers CEA and TIMP-1 increase in the case of disease and therefore can be used significantly in detection and the diagnosis of the disease early and improve its treatment and recovery from it
Key Words: CEA, TIMP-1, CRC.

Introduction
One of the most common human diseases associated with the presence of a tumor is colon and rectal cancer, and in addition to that it causes death to a very large number of people worldwide[1]. The arrangement of colorectal cancer among the most common cancers is the third after the cancers that spread and infect the lung, as well as those that affect the breast worldwide. It appears clearly that it spreads huge and wide, and all types of colon and rectal cancer constitute two thirds of all these type[2].

Among the tools that help to detect and diagnose colorectal cancer and contribute significantly to improving the rate of discovery is colonoscopy, which is known to be very tired, unpleasant, irritating, and uncomfortable for many people in addition to being very expensive in the case of using it to know the conditions associated with the disease and its symptoms with all patients. Therefore, it is one of the required tasks it is necessary to search and identify new diagnostic signals that help early and very quickly to detect colorectal cancer.

A lot of valid studies have been found and have a close relationship in this regard, where research and guidelines have been conducted to determine the protein markers of many types of cancers, the most important are linked to colorectal cancer[3].

Among the protein markers is Vascular Endothelial growth factor (VEGF) which called also vasculotropin, it is consists of a dimeric heparin-binding glycoprotein, has a molecular weight of 45- K Da and it has four isoform, It is present and constructed in many natural tissues in humans, but if its expression is clearly and significantly found, that indicate it is a sign of a large potential for tumor growth and the possibility of spread by angiogenesis activation.[4]. Therefore, it has been suggested that it have a significant impact on the development of the tumor, especially at the beginning of the formation of the adenoma, in addition to playing an important role in the spread of the disease, due to metastatic spread[5].

And also Urokinase-type plasminogen activator (u-PA) is one of the factors which is a 55-kDa enzyme and that key for metastasis and has an important role in the spread and expansion of the tumor, because it is made inside many different types of cells Among the factors that are able to operate a chain of proteinases implicated in extracellular matrix degradation [6], [7].

Another known protein marker is Carcinoem-bryonic antigen (CEA), is classified an onco-fetal glycoprotein, before birth its production process stops, CEA approximately 180-kDa and a member of immunoglobulin cell adhesion molecules superfamily (CAMs) , It is produced naturally during the growth of the fetus in gastrointestinal tissue [8].

It binds strongly to the tumor cell by attaching to its own plasma membrane and excreting from it to the blood. In the case of colorectal cancer massive quantities expressed from it, but it is highly expressed by natural mucosal cells in cases of Adenocarcinoma [9].

Excessive expression of the CEA and persistent release of tumor cells often leads to metastatic spread and this supports tumor formation due to

the presence of the inflammatory environment [10]. And when a tumor becomes enlarged CEA accumulates and appears significantly in the blood which lead to support its use in monitoring and evaluating the increasing activity of tumors, especially malignancy and extremely useful in case of follow-up so the CEA recommended in both American and European guidelines[11].

Also, Tissue inhibitor of metalloproteinases-1 (TIMP-1) affecting the regulation of cell growth and degradation, It is a glycoprotein expressed by the cells of multiple tissues of humans belongs to TIMP family which are group of proteins that inhibit matrix metalloproteinase (MMPs), This group is known as peptidases that has a major role in both apoptosis in non-nervous tissues, degradation of the extracellular matrix, proliferation, migration and   regulate cell growth[12]. In particular TIMP-1 has a big role more of any other factor in the development, growth and spread of the tumor and There is a relationship with a significant difference between the development of the tumor and the level of concentration of TIMP-1 in the blood and accompanying it poor patient prognosis and this has been mentioned this and it has been proven before in many clinical studies[13,14]. However And we still have a great need to study TIMP-1 and its effect and how it plays an important role in the development of cancer, Also,[15] Significantly, regulation of TIMP-1 was found in the tissues of colon and rectal cancer samples, compared to normal tissues.

Material and Methods
Eighty-four Egyptian patients were enrolled in the study of tumor makers of CRC which are CEA and TIMP-1. They were categorized into 64 patients with CRC: males (45.5%) and females (54.5%) with age range from 23 to 73 years (Mean ± SD: 45.8 ± 12.4 years) and 20 healthy controls: 11 males (55%) and 9 females (45%) with age range from 25 to 67 years (43.7 ± 10.8 years). All samples were collected from Mania National Oncology Center.

This study passed **the criteria for exclusion and criteria for selection** for the patients that entered in the study. **The selection criteria included the following:**
1- Egyptian nationals only.

Investigating The variations in The expression level of CEA and TIMP-1 markers in Human

2- Age not less than 18 years when the sample is withdrawn.
3- Only patients with colon and rectal cancer.
4- The volunteer's consent to enter the study And his health condition allows taking the blood sample from him.
5- Proving and diagnosing the disease by other methods than this analysis included in the study.
6- Completion of the information in the patient's file.

As for exclusion criteria, the following were excluded:
1- patients other than Egyptian nationality, where we found Syrian, Libyan, and other nationalities during the study.
2- patients those under 18 years of age.
3- patients whose health condition did not allow him to withdraw the blood sample from him.
4- patients with other types of tumors and the patient who have more than one type of cancer, even among them, colon cancer.
5- the absence of some or all of the information in the patient's file .

They were Egyptian patients who were subjected to the following: full history taking, clinical and laboratory examinations including measurement of liver transaminases (ALT and AST). Patients and controls were matched for age, gender, presence of previous hepatic viral infection, bilharziasis, hypertension, smoking and pills.

The analyses were carried out in Mania University, Faculty of Pharmacy, and Laboratory of Biochemistry Department; and in Deraya University, Faculty of Pharmacy, and Laboratory of Biochemistry Department. The Research Ethics Committee of the Faculty of Pharmacy, Mania University, gave clear approval to the experiment, and each patient who was enrolled in this study was given written consent and informed consent.

CEA Immunoassay
The Can Ag CEA ELISA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. The CEA ELISA kit was purchased from Clinilab Co., Can Ag CEA Catalog# EIA REF401-10, and the procedures were performed as recommended by the

manufacturer. Calibrators, controls and patient serum samples are incubated with biotinylated

Anti-CEA monoclonal antibody and horseradish peroxidase (HRP) labeled Anti-CEA monoclonal antibody in streptavidin coated microstrips. After washing, buffered substrate/ chromogen reagent (hydrogen peroxide and 3, 3', 5, 5'-tetra-methylbenzidine) is added to each well and It is allowed to proceed with an enzyme reaction. When an enzyme reaction occurs, it will produce a blue color, which
indicates the presence of the antigen and after that the absorbance is measured at wavelength 620 nm and that when the color development is stopped.

TIMP-1 Immunoassay
This assay employs the quantitative sandwich enzyme immunoassay technique. The TIMP-1 ELISA kit was purchased from Clinilab Co., Human TIMP-1 Immunoassay Catalog# DTM 100, and the procedures were performed as recommended by the manufacturer. A monoclonal antibody specific for human TIMP-1 has been pre-coated onto microplate wells. Standards and samples are pipetted into the wells and any TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, HRP-linked polyclonal antibody specific for human TIMP-1 is added to the wells. After incubation and washing, a substrate solution is added to the wells and color develops in proportion to the amount of TIMP-1 bound in the initial step. The color development is stopped and the absorbance is measured at wavelength 405 nm.

Statistical Analysis
The collected data were statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 21. Descriptive statistics were expressed for parametric quantitative data by mean and standard deviation, while they were presented for categorical data as number and percentage. Analyses were done for parametric quantitative data between the two groups by T test and among the three genotypes using One Way ANOVA test followed by Duncan test between each two genotypes. Analyses were done for qualitative data using Chi-square test (if the number per cell more than 5) and Fisher Exact test (if the number per cell less than 5). The

level of significance was taken at (P value < 0.05).

Results

The present study is a prospective study included a total of 84 subjects with age range of 23:73 years who were allocated to two groups:

❖ **Group (I): Patients:** included 64 CRC patients.

❖ **Group (II): Control:** included 20 healthy control subjects.

Table (1) : Demographic data between studied groups.

Variable		Groups		p.value (Sig.)
		Patients (n=64)	Control (n=20)	
Age (year) mean ± SD, range		45.8 ± 12.4 (23-73)	43.7 ± 10.8 (25-67)	0.498NS
Sex	Males	29 (45.3%)	11 (55.0 %)	0.499NS
	Females	35 (54.7%)	9 (45.0 %)	
Residence	Rural	31 (48.4%)	8 (40.0%)	0.509NS
	Urban	33 (51.6%)	12 (60.0 %)	
Job	Did not work	15 (23.4%)	6 (30.0%)	0.037*
	Farmer	11 (17.2%)	4 (20.0%)	
	House wife	29 (45.4%)	2 (10.0%)	
	Driver	2 (3.1%)	2 (10.0%)	
	Sales	1 (1.5%)	0	
	Worker	6 (9.4%)	6 (30.0%)	
Smoking	No	48 (75.0%)	13 (65.0%)	0.381NS
	Yes	16 (25.0%)	7 (35.0%)	

Independent sample T-test and chi-square test were used.
* Significant (P≤ 0.05) (NS) Not significant

Comparison between laboratory investigations of studied groups
Statistical analysis of the data of the disease historical report showed the presence of significant differences between patients group and control group in Total Bilirubin, alpha-feto protein (AFP) and (C A 19-9) markers .

Table (2): Laboratory investigations between studied groups.

Variable	Groups		p value
	Patients (n=64)	Control (n=20)	
ALT (U/L)	34.6 ± 24.4	32.4 ± 10.8	0.697[NS]
AST (U/L)	40.8 ± 21.8	38.7 ± 14.2	0.686[NS]
T. Bilirubin (mg/dL)	2.29 ± 1.84	1.21 ± 0.31	0.013*
Albumin (mg/dL)	3.78 ± 0.71	3.84 ± 0.62	0.734[NS]
Glucose (mg/dL)	123.1 ± 64.9	118.4 ± 10.7	0.748[NS]
Urea (mg/dL)	29.2 ± 16.3	28.1 ± 11.8	0.780[NS]
Creatinin (mg/dL)	0.68 ± 0.28	0.57 ± 0.15	0.101[NS]
Hemoglobin (%)	12.5 ± 2.1	13.1 ± 1.9	0.060[NS]
AFP (ng/ml)	0.99 ± 0.45	0.21 ± 0.09	<0.01**
CA 19-9 (U/mL)	38.1 ± 11.6	14.2 ± 7.8	<0.01**

❖ Independent sample T-test was used.
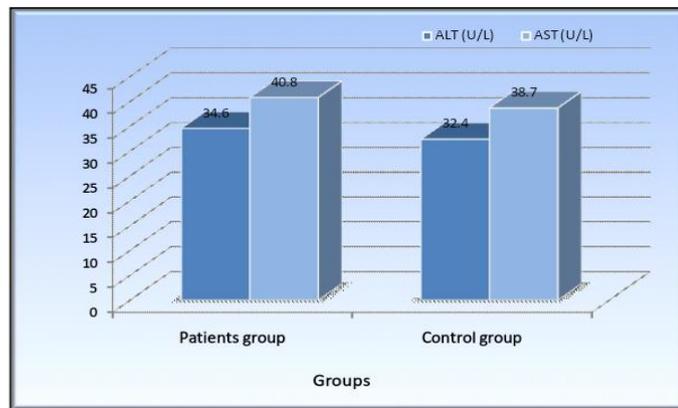❖ NS: not significant,*: significant ($p \leq 0.05$), ** significant ($p \leq 0.01$).

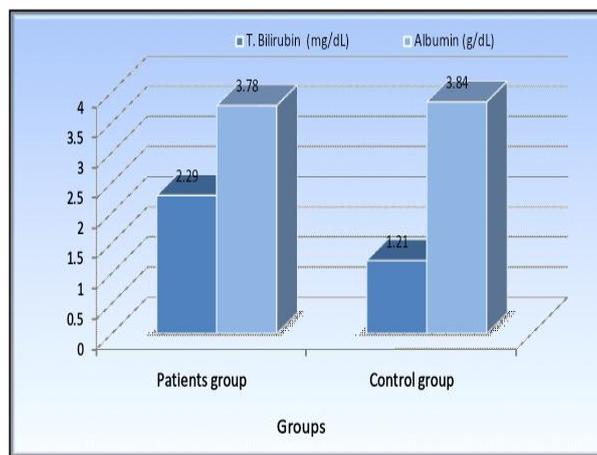**Figure (1):** AST and ALT level between groups.

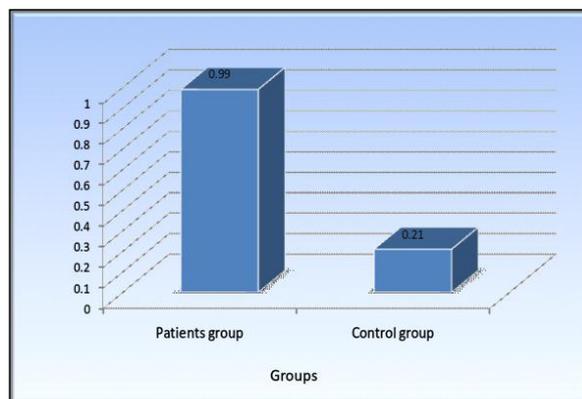**Figure (2):**T. Bilirubin and albumin level between groups.
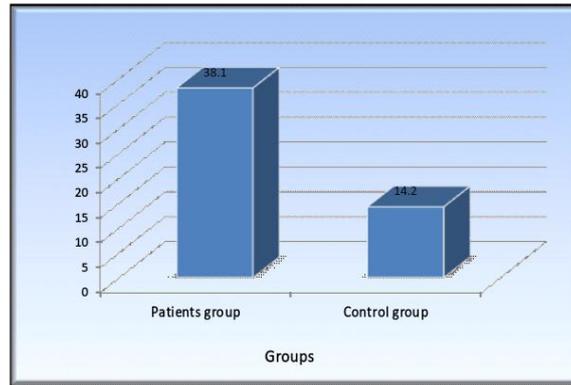

Figure (3): AFP level between groups.

Figure (4): CA 19-9 level between groups.

Table (3): Family history and grade of Adenocarcinoma of patients group.

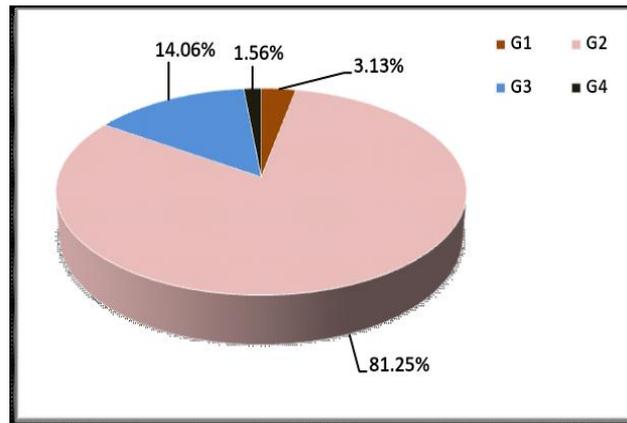| Variable		Description(n=64)
Family history of Adenocarcinoma	**No**	(100.0%)
	Yes	0
Grade of Adenocarcinoma	**G1**	2 (3.1%)
	G2	52 (81.3%)
	G3	9 (14.1%)
	G4	1 (1.5%)

Figure (5): Grades of adenocarcinoma in patients group

Comparison between groups regarding plasma level of CEA
Our ELISA results indicated a significant increase in plasma level of CEA in patients of CRC compared to the control group.

Table (4): Comparison between groups regarding plasma level of CEA.

Variable	Groups		p value
	Patients (n=64)	**Control (n=20)**	
CEA (mg/L)	0.51 ± 0.74	0.22 ± 0.11	**0.008****

❖ Independent sample T-test was used.
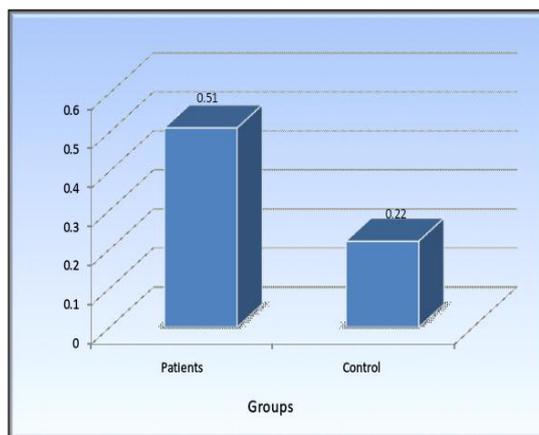❖ ** Significant ($p \leq 0.01$).

Figure (6): Comparison between groups of CEA level in plasma.

Comparison between groups regarding plasma level of TIMP-1

Our ELISA results indicated a significant increase in plasma level of TIMP-1 in patients of CRC compared to the control group.

Table (5): Comparison between groups regarding plasma level of TIMP-1.

Variable	Groups		*p* value
	Patients (n=64)	Control (n=20)	
TIMP-1 (ng/ml)	0.25±0.16	0.14± 0.11	**0.005****

❖ Independent sample T-test was used.
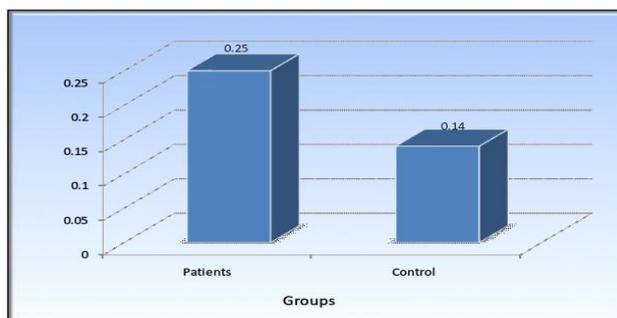❖ ** significant ($p \leq 0.01$).

Figure (7): Comparison between groups of TIMP-1 level in plasma.

Discussion

In this current study, the objective is to diagnose and determine the plasma level of certain tumor markers of CRC to use them in early diagnosis of CRC.

It is known that CRC causes large percentage of deaths worldwide, and this rate is estimated annually, according to [1]. In Egypt, CRC represents 3% of all malignant tumors (excluding tumors of the nervous system)[16].

The correct early diagnosis of the disease leads to non-exacerbation or occurrence of secondary symptoms as a result of the disease and it is very much required and necessary to avoid the secondary complications of the disease. Thus, it

helps us determine the appropriate treatment quickly and accurately.

In this particular disease, CRC must be diagnosed quickly to deal with and succeed in eliminating it because it is very serious and lethal.

There are many ways to diagnose CRC through the use of different modern types of radiant rays as X-rays, the symptoms, devices such as colonoscopy and the analysis.

The current methods of diagnosis, which are widely used, are the use of analysis of some signs of tumors, which are linked to certain type of cancer and indicate its presence in the body or the location of the affected organ.

We found that laboratory investigations showed significant differences between patients group and control group in both of T. Bilirubin and AFP as shown in Table (2).

Each type of cancer has known specialized signs and may have one or more specific markers. Thus, the presence of specific markers in a high level may reflect the presence of certain type of cancer in this patient.

Some markers that increase the incidence of CRC include: Vasculotropin also known as VEGF[4], U-PA[7], CEA[8], TIMP-1[15[, Cell-surface markers for colon cancer (CLDN1, GPR56, GRM8, LY6G6D/F, SLCO1B3 and TLR4) [17], Molecular markers for colon cancer (BRAF V600E, KRAS, MSI, PI3K and TP53) [18].

And looking in the patients that samples were withdrawn from them and entered in to the current study and following up on their clinical file showed us that most of the cases were diagnosed in the second grades of adenocarcinomas and treatment was started inside the oncology center, and this means that the flashing occurred not so long ago which confirms and indicates. The presence of markers with a clear increase during the withdrawal of samples, as shown in the Table (3) and figure[5].

In this study, we studied two of the markers of colon cancer, CEA and TIMP -1, and verified their level in plasma by ELISA technique.

We found a significant increase in the level of each of CEA in patients group compared to control group as shown in Table (4).

In this study, it was confirmed that the level of CEA in blood plasma is high in CRC patients and it can be used to early diagnose the disease with the help of other diagnostic tools. This is in agreement with previously published studies [19-21].

Regarding TIMP-1 marker, there was also a significant increase in the blood plasma level of TIMP-1 in CRC patients compared to normal control cases as shown in Table (5).

Therefore, this study also confirms that the level of TIMP-1 marker increases in cases of colon cancer. This is in agreement with previously published studies [22].

We can use both markers to predict the disease development and we can analyze their presence together in predicting and verifying the presence of CRC and determining its degree and severity [23].
We conclude that both CEA and TIMP-1 are markers in early correct diagnosis of CRC.

The study has some limitations of these the relatively small sample size and we recommended further larger studies to confirm our findings.

Disclosure Statement
The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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