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Study of biomarkers in colorectal cancer invasion to the liver

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Abstract: Objective: Colorectal liver metastases (CRLM) is the preferred target site for CRC distal metastasis, which is always a cause for high mortality rates. Additionally, metastasis is frequently accompanied by low survival rates and renders standard therapies ineffective. Finding markers for CRLM identification is thus crucial for the early detection of CRLM. **Methods:** In order to validate our idea, we recruited patients with CRC. Ninety-six human participants were chosen along with a case-control study. The participants were divided into 3 groups: group 1 contains 62 CRC patients, group 2 contains 34 CRLM patients, and group 3 contains 80 healthy people. Bilirubin, SGOT, SGPT, CEA, and CA19.9 were investigated in all participants. **Results:** In comparison to control persons, CRC patients' serum levels of CEA and CA 19-9 were considerably higher. When CRLM patients were compared to non-distant metastatic patients, CEA and CA19.9 tumor markers showed significantly higher levels. **Conclusion:** Our findings showed that serum levels of CEA and CA19.9 are useful diagnostic markers for CRC for detection of metastasis using disturbed liver functions as a correlative indicator.

Keywords: Carcinoembryonic Antigen (CEA), Colorectal Cancer (CRC), Colorectal Liver Metastasis (CRLM), Cancer Antigen 19-9 (CA 19.9).

1. INTRODUCTION

The third most fatal disease in the world, colorectal cancer (CRC), is regarded as one of the most common malignancies in the world. Adaptive intra-tumor immunity may help the tumor either disappear or grow[1]. CRC is regarded as the second most deadly disease, believed to be responsible for almost 935,000 cancer deaths, and it accounts for approximately 1.9 million new instances of epithelial malignancy globally, ranking third[2].

Up to 50% of individuals with this condition will eventually develop liver metastases throughout the course of their illness[3]. CRC accounts for almost half of all gastrointestinal tract malignancies in Egypt, ranking ninth among females and tenth among males[4].

Relating to Cairo University's National Cancer Institute registry, Egypt has a high prevalence of CRC, with a positive diagnostic of CRC being found in 14.0% of all colonoscopies[5]. 90% of all fatalities connected to cancer are caused by metastases. The liver is the well-known target organ for all malignant processes, and the lungs(10–20%) and liver(70%) are the most typical targets for CRC metastases. Liver metastases are evident at the time of diagnosis in 20–25% of CRC patients (synchronous metastases). The same percentage of patients will get metastases (also known as metachronous metastases) two years after the initial tumor has been removed[5-6]. Unfortunately, roughly 20% of CRC patients already have metastatic disease when they first appear, which dramatically lowers the 5-year survival rate from 90% to 12%. Prevention of its development into the metastatic condition is crucial because of this[7]. Tumor biomarkers are used in illness diagnosis, prognosis, therapeutic response monitoring, and disease recurrence prediction. It is widely acknowledged that by accurately diagnosing and predicting early-stage illness, diagnostic and prognostic indicators may reduce cancer-related mortality. The identification of cancer biomarkers is one of the most promising methods for the diagnosis

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of early stage malignant or even pre-malignant lesion using a simple blood test[8]. Predictive markers also assist in predicting the patient response to therapy[9].

A patient-accepted, noninvasive method may be used to draw peripheral blood. As a result, the calculation of serum tumour biomarkers complied with the standards of tumour specificity and the noninvasive examination concept. The immunoglobulin super family of cell adhesion proteins includes the complex glycoprotein of the membrane surface known as carcinoembryonic antigen (CEA). The most often utilised biomarker for detecting CRC and assessing prognosis or cancer recurrence after therapy is CEA[10], accordingly Advanced CRC patients should be monitored using CEA, particularly if metastases is difficult to detect with other techniques[11].

An example of a type 1 terminal carbohydrate structure is the cancer antigen 19-9, which is crucial for cell adhesion, local tumour invasiveness, and metastasis[12]. It is one of the most widely utilised tumour biomarkers in gastrointestinal malignancies and was developed against a colon carcinoma. It is used to identify a monosialoganglioside present in individuals with gastrointestinal adenocarcinoma. It is released by healthy biliary epithelium, and benign biliary illness and inflammation may significantly raise its level[13–14]. About 21%-42% of patients with gastric cancer, 20%-40% of patients with colon cancer, and 71%-93% of patients with pancreatic cancer had increased CA19-9 levels[15].

This study aims to assessing the blood levels of CEA and CA19.9 in preoperative CRC patients and CRLM patients is the initial goal of our research.

As CRLM is typically associated with a poor prognosis and decreased overall survival, our study sought to ascertain whether preoperative serum CEA and CA19-9 levels have a prognostic value for identifying recurrence of colon cancer and predicting distant metastasis to liver.

2. METHODS

2.1. Individuals

Our research is a case-control study. Between 2019 and 2020, the colorectal surgery department and the medical oncology sections at Mansoura University Hospital in Egypt recruited 96 human volunteers from both inpatient and outpatient patients. All of our participants gave written informed permission for our study, which was conducted in conformity with the World Medical Association's Declaration of Helsinki. The Independent Ethics Committee of the Institutional Review Board of the Faculty of Medicine at Mansoura University in Egypt and the Independent Ethics Committee of the Faculty of Pharmacy for Girls at Al Azhar University in Egypt both approved the experiments (IRB no. R/17, 11, 75 and R/10, 60, respectively).

Among all studied cases, 47.9% (46/96) of cases had tumor located in colon, and 56.1% (50/96) had tumor located in rectum, tumor size median value was 6 cm (range 1.4 -6.3), non-distant metastasis cases (stage 1-3) were 62 case, and distant metastasis cases were 34case.

Inclusion Criteria:

This study will include patients fulfilling the following criteria:

1. Age: > 18 years old.
2. Gender: Both male and female.

Exclusion Criteria:

Patients suffer from other inflammatory or cancer disease.

2.2. Research plan

According to fissions, CRC was diagnosed Based on histological analysis of biopsy samples and clinical staging tests, patients and international guiltiness were divided into two groups: CRC patients without distant metastasis and colorectal liver metastasis (CRLM)[16]. The research population was divided into three groups: Group I included 62 CRC patients without distant metastases, Group II included 34 CRLM patients, and Group III included 80 healthy people as the control group. Age, sex, and BMI were all matched across all study groups (Table 1).

2.3. Sample gathering

Under strict aseptic conditions, ten milliliters of fresh blood were collected from each patient. For CBC testing, two milliliters of blood were put in a tube containing K2EDTA. The remaining blood was placed in sterile vacutainers and allowed to coagulate for 30 minutes. Centrifuge for 15-minute on at 1000 g. Separate the serum into two portions, one of which was utilized for the immediate measurement of standard laboratory parameters and the other of which was kept at 20 °C for later use in the measurement of CEA and CA19-9.

2.4. Biochemical evaluation

CBC was estimated (BY Sysmex kx-21 N heamatology analyzer.) and serum levels, as well as the activities of bilirubin, SGOT, and SGPT, were estimated using commercial kits in accordance with the manufacturer's recommendations. Following the manufacturer's instructions (By cobas c 311 analyzer), serum levels for CEA and CA19-9 were

determined using ELISA kits from Monocent, Inc. (ref: EL1-1283 and EL1-1280, respectively).

2.5. Statistical investigation

The (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) programme was used to analyse the data. Standard deviation and mean values were used to represent the data. T-Student test was used to evaluate significant differences between groups. The Pearson's correlation coefficient was used to examine the link between continuous variables. From the associated ROC curves, the sensitivity, specificity, and diagnostic precision of CEA and CA19.9 were determined. The closest point to the curve's top left corner was chosen as the ideal cut off value (the highest point of the plot).

Sample size calculation:

Sample size calculation was based on difference of CEA between cases with CRC, CRLM, & control groups retrieved from previous research (Kim et al., 2017). Using G*power version 3.0.10 to calculate sample size based on difference of expression 18%, 2-tailed test , α error =0.05 and power 80.0% then total sample size will be 80 at least in each group. Sample size was also claculated on CA19.9 and was found to be 30 in each group then sample size calculation was based on CEA.

3. RESULTS

3.1. Baseline information and laboratory evaluation

The CRC group had reduced haemoglobin levels and platelet counts ($p<0.001$ and $p<0.01$, respectively). WBC count, bilirubin, SGOT, SGPT, and creatinine serum levels, on the other hand, showed no significant changes between the CRC group and the controls. When compared to control persons, CRC patients had substantially higher serum levels of CEA and CA 19-9 ($p<0.001$ for each) (Table 1).

3.2. Correlations between phases and examined laboratory parameters

Age, gender, and BMI were the same for the CRLM and non-distance metastasis groups in both. Comparing CRLM patient group (stage 4, n=34) to non-distant metastasis patient group (stage 1-3, n=62), CRLM patient group revealed significantly reduced platelet account and significantly higher total serum bilirubin level, SGOT and SGPT serum levels ($p<0.001$for each). Additionally, distant metastatic patients had significantly higher levels of the tumour markers CEA and CA19.9 than non-distant metastatic patients ($p<0.001$ for each). Other than that, there were no discernible differences between CRC patients with and without liver metastases in terms of other laboratory markers (Table 2).

3.3. Study of Correlation

Table 3 presents the results of a statistical analysis of the correlation research between CEA and CA19.9 and other investigated parameters. This correlation analysis found a substantial positive link between each marker and each stage of the illness ($r=0.696$, $p<0.001$; $r=0.337$, $p<0.001$, respectively), but not between them and the other examined parameters (Table 3). (Figure 1 and 2)

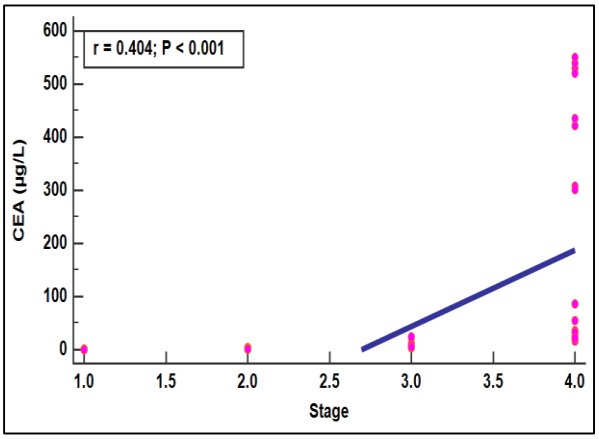


Figure 1. Scatter plot of correlation between stages and CEA level

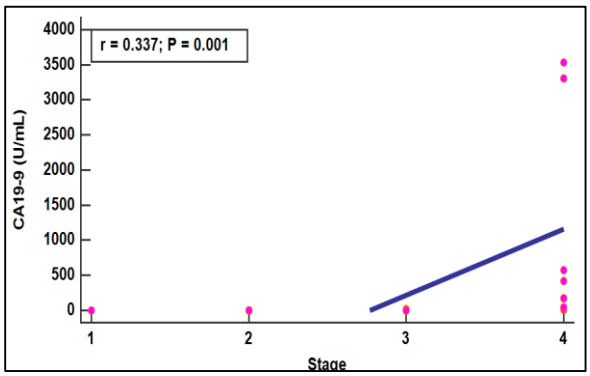
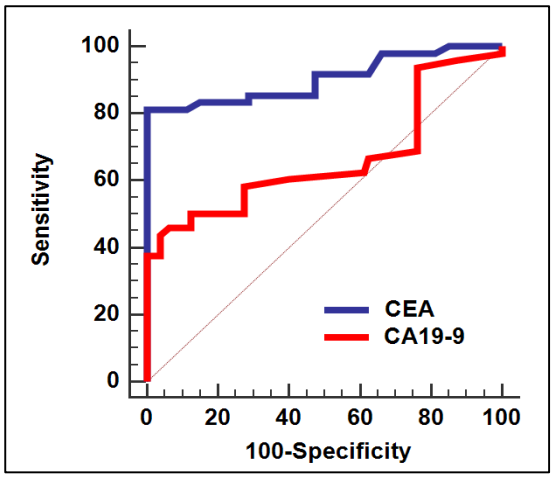


Figure 2. Scatter plot of correlation between stages and CA19-9 level.

3.4. Validity research for the CEA and CA19.9

In order to establish a cut-off value that distinguishes CRC patients from healthy participants and to assess each of their diagnostic accuracies, the serum levels of CEA and CA19.9 were statistically examined. Excellent AUC (0.904, 95% Cl 0.858-0.950), accuracy (84.4%), and high sensitivity (83.3%) and specificity (85%) were all shown by the ROC curve that recognised the CEA

level at the cut point of 2µg/L. While CA19.9 serum level shown low AUC 0.663, 95% Cl 0.581- 0.744), accuracy (64.8%), sensitivity (58.3%), and specificity (72.5%), it also displayed poor sensitivity and accuracy. Additionally, the ROC curve of CEA and CA19.9 was used to separate CRLM patients from non-distant metastatic cases. With 100% sensitivity and 96.8% specificity for CEA and 94.1% sensitivity and 100% specificity for CA19.9,



respectively, both markers had good AUC (0.988, 95% Cl 0.971-1; 0.992, 95% Cl 0.980-1). In (Table 4), performance characters are shown (Figure 3 and 4).

Figure 3. ROC curve of CEA and CA19-9 for discrimination between cases and controls.

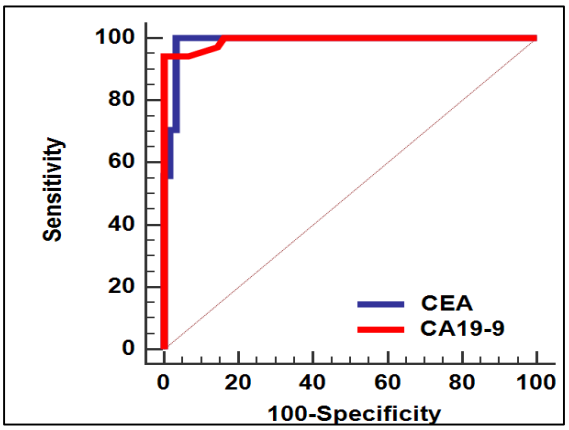


Figure 4. ROC curve of CEA and CA19-9 for discrimination between metastatic and non metastatic CRC case.

Table 1: Demographic and laboratory data among CRC and control group, and Association of stages with studied parameters:

		Control		CRC		*P*	Stages				
		n=80		n=96			Non metastatic		Metastatic		*P*
		Mean /count	SD/%	Mean/count	SD/%						
Age (years)	mean±SD	55	±10.3	56.8	±9.6	0.385	55.5	10.7	59.0	6.7	0.090
Male	N, %	26	32.5%	44	45.8%	0.172	24	38.7%	20	58.8%	0.059
Female	N, %	54	67.5%	52	54.2%		38	61.3%	14	41.2%	
BMI (kg/m2)	mean±SD	30.3	±5.4	30.1	±5.4	0.818	30.1	5.5	30.0	5.2	.947
WBC ($X10^9$/L)	mean±SD	8.0	1.7	7.0	4.1	.221	6.2	3.6	5.7	4.0	0.187
Hb (g/dL)	mean±SD	14.1	1.1	10.9	2.2	<0.001	10.9	2.0	10.9	2.5	.967
PLT ($X10^9$/L)	Median, range	285.0	156-446	227.5	42-694	.005	274.0	164-694	66.0	42-480	<0.001
Total bilirubin (mg/dL)	Median, range	0.6	0.5-1.1	0.8	0.3-4.7	.254	0.6	0.25-1.76	2.3	0.5-4.7	<0.001
SGOT (U/L)	mean±SD	27.8	3.7	29.0	13.0	.369	23.6	9.2	38.7	13.4	<0.001
SGPT (U/L)	mean±SD	26.5	3.3	28.2	16.3	.433	20.4	7.8	42.5	17.9	<0.001
SrCr. (mg/dL)	Median, range	0.9	0.6-1.1	0.8	0.5-2.7	.239	0.8	0.6-2.7	0.9	0.5-1.2	.864
CEA (µg/L)	Median, range	1.3	0.1-2.4	7.7	0.5-550	<0.001	4.6	0.5-25	33.0	16.9-550	<0.001
CA19.9 (U/mL)	Median, range	5	1-34	7.2	0.6-3535	<0.001	3.0	0.6-22.5	42.6	12-3535	<0.001

BMI: Body Mass Indix, WBC: White Blood Cell, Hb; Hemhlobin, PLT: Platelet Count Test, SGOT: serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, SrCr: Serum Creatinine, CEA: Carcinoembryonic antigen , CA19.9: Cancer antigen 19-9.

Table 2: Correlation of CEA and CA-19-9 with other parameters:

	CEA		CA19-9	
	Rs	***P***	***rs***	***P***
Age	.158	.137	.143	.159
BMI (kg/m^2)	-.104	.172	-.103	.175
Tumor size (cm)	-.053	.611	-.035	.733
Stage	.404	<0.001	.337	.001
WBC (X10^9/L)	-.055	.472	.013	.860
Hb (g/dL)	-.102-	.107	-.080	.291
PLT (X10^9/L)	-.168	.126	-.182	.116
Total bilirubin (mg/dL)	.169	.125	.173	.122
SGOT (U/L)	.176	.120	.179	.117
SGPT (U/L)	.039	.612	.042	.578
Sr Cr. (mg/dL)	.102	.104	.128	.198
CEA (µg/L)			.696	<0.001
CA19.9 (U/mL)	.696	<0.001		

BMI: Body Mass Indix, WBC: White Blood Cell, Hb; Hemhlobin, PLT: Platelet Count Test, SGOT: serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, SrCreatinine: Serum Creatinine, CEA: Carcinoembryonic antigen , CA19.9: Cancer antigen 19-9.

Table 3: Biomarkers validity measurements of CRC patients groups:

Group	Marker	AUC	95% CI	cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Cases And Controls	CEA (µg/L)	0.904	0.858 - 0.950	2	83.3	85	75.3	90.3	84.4
	CA19-9 (U/mL)	0.663	0.581 -0.744	6	58.3	72.5	71.8	59.2	64.8
Non-CRLM and CRLM	CEA (µg/L)	0.988	0.971 -1	10.8	100	96.8	94.5	100	97.9
	CA19-9 (U/mL)	0.992	0.980 -1	22.5	94.1	100	100.0	96.9	97.9

CRLM: Colorectal Liver metastasis, AUC: area under ROC curve; PPV: positive predictive value, NPV: negative predictive value, OR: odds ratio, CI: confidence interval.

4. DISCUSSION

The discovery of molecular markers may speed up CRC disease diagnosis and therapy. Serum protein indicators have great promise for the creation of CRC detection assays that are accurate, non-invasive, and affordable. A patient's biomarker profile, which includes a number of distinct biomarkers, may provide greater diagnostic precision than a single marker[17].

Our study's key results concur with those of other research on CRC patients, which found that these patients had significantly higher blood levels of CEA and CA19 -9 compared to the control group[17–18]. After evaluating the data, we discovered that patients had substantially higher median levels of CEA and CA 19-9 (p<0.001for each) and CRLM (p<0.001 for each) before surgery as compared to control individuals and non-liver metastasis cases, respectively. Additionally, correlation statistical analysis revealed a strong positive association between CEA and CA19-9 (r=0.69: p<0.001 for each) as well as between the median blood levels of CEA and CA19-9 and disease stages (r=0.404: p0.001 and r=0.337: p<0.001, respectively). This is also in line with Yanfeng Gao and his colleagues'(2018)[16] report, which stated that CEA and CA19-9 can be used as diagnostic biomarkers and that the ability to predict CRC metastasis depends on the positive correlation rates of each of these markers through the stages of stage 1-3 (non-

distant metastasis) and stage IV (distant metastasis to liver). Our findings were analogous to those of Meteoglu and Coworkers (2008)[19], who discovered that serum CEA and Dukes stage in colorectal cancer were closely associated. According to a linear link between cell quantity and blood CEA, tumour cells produce CEA. In agreement with our findings, Wenhao Zhou and his colleagues (2019)[20] found that patients with a high CA19-9 level had a considerably higher risk of metastasis than those with a low CA19-9 level. Cellular adhesion is facilitated by the cell surface glycoprotein known as CA19-9. Cancer cells that express CA19.9 have a higher capacity for invasion and a higher propensity for metastasis[21–22]. According to a prior research, preoperative CA19-9 levels may aid in the prediction of distant metastasis, which is consistent with our findings. Results demonstrated that high CA19-9 levels induce a threefold greater cumulative incidence of distant metastasis compared to low CA19-9 levels[23–24], which is linked with an elevated risk of distant metastasis (liver and/or lungs).

Contrary to our findings, serum CA 19-9 was observed in the cancer group's patients compared to the control group; however, no significant link with disease stage was discovered[25]. Previous studies showed that the tumour marker CA19-9 was sensitive and its level rises as the illness progresses with no connection to tumour staging[26–27]. This variation may result from the different traits of colon cancer patients with varied degrees of danger. Patients with advanced illness were often more likely to have aggressive tumours, a high tumour load, and worsened immunosuppression[28-29.] Serum CA19-9 levels have previously been demonstrated to properly reflect tumour metabolism and tumour load, making them potentially useful for tracking therapy response and cancer progression as well as more sensitive for predicting survival outcomes in patients with high-risk colon cancer[25,30].

The determination of the predictive value of the selected indicators for diagnosis was done using validity measurement. In comparison to CA19.9 at the best cut off point > 6 U/mL (AUC= 0.663; 95% Cl: 0.581 -0.744, Accuracy= 64.8), serum markers levels identified that cut-off value of CEA serum levels > 2 µg/L showed excellent AUC (0.90; 95% Cl: 0.858-0.950) and high Accuracy (84.4%). Additionally, at corresponding cut off points, CEA reported higher sensitivity and specificity (These findings are consistent with Yanfeng Gao and his colleagues' findings from 2018[16], which claimed that CEA has the greatest sensitivity compared to CA19.9, however our findings claimed that CEA has the lowest specificity. Additionally, CEA has been demonstrated to have the greatest ROC curves for the measured parameters for the diagnosis of colorectal cancer among other tumour markers. The closest to the ideal sensitivity and specificity is CEA, which has the largest area under the curve[31].

Both CEA and CA19-9 in our study reported excellent AUC (0.988, 95% Cl: 0.971-1;0.992, 95% Cl: 0.980-1, respectively) and high accuracy (97% for each), with high sensitivity(100%,96.8%, respectively) and specificity (94.1%,100%), respectively, as predictive markers for distant metastasis to liver. In accordance with our findings, earlier research revealed that CA19-9 is regarded as a predictive factor for CRC patients who had high baseline CA19-9 levels. They might have a significant risk of postoperative metastasis, which would lower their chance of survival[32–33]. One group of cell surface glycoproteins includes CA19-9. It's necessary for cellular attachment. Cancer cells that express CA19-9 may be more invasive and capable of metastatic spread[20,22]. Additionally, the CA19-9 monosialoganglioside may contribute to metastasis in CRC by inducing platelet aggregation in tumour cells. Due to CA19- 9's role in carcinogenesis, its malignant nature and increased levels may be a factor in tumour spreading and treatment resistance[34]. Additionally, liver metastases and increased CEA levels are related. As a predictive and diagnostic tumour marker in cancer patients, CEA is often employed. It impacts It prevents the death of circulating cancer cells and binds to heterogeneous nuclear RNA binding protein M4, a protein that is a receptor for Kupffer cells. This activation of Kupffer cells causes them to secrete various cytokines that alter the microenvironment and promote the survival of colorectal cancer cells in the liver. Additionally, it makes cellular adhesion molecules active[35].

5. CONCLUSIONS

A biologically aggressive illness was suggested by high blood levels of CEA and CA19-9. CEA is more sensitive and specific than CA19-9, but both have a high sensitivity and specificity for predicting CRC liver metastasis. According to their levels, both CEA and CA19-9are useful together in confirming the diagnosis of CRC. Additionally, their blood levels may be utilized to monitor and manage the disease's severity and the emergence of liver metastases. Many anticancer medications have proved the strong association between CEA and CA19-9 metastasis and response to cancer therapy. The development of anticancer strategies for the treatment of colorectal cancers will have a significant probability of success if the precise involvement of CEA and CA19-9in CRC and CRLM is understood.

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Conflicts of Interest: The authors declare that they have no competing interests.

Ethical Statement: All of our participants gave written informed permission for our study, which was conducted in conformity with the World Medical Association's Declaration of Helsinki. The Independent Ethics Committee of the Institutional Review Board of the Faculty of Medicine at Mansoura University in Egypt and the Independent Ethics Committee of the Faculty of Pharmacy for Girls at Al Azhar University in Egypt both approved the experiments (IRB no. R/17, 11, 75 and R/10, 60, respectively).

Author Contribution: Enas Y Abdel-Hamid.; Investigation, Methodology, Resources, Writing Ogiginl draft. Nabil M Abdel-Hamid.; Edit manuscript. Rehab R El-Awady.; review the manuscript and editing it. Nahla Anber.; Supervision of the practical part.

List of Abbreviations: CRC: Colorectal cancer; CEA: Carcinoembryonic antigen; CA19-9: Cancer antigen 19-9; CRLM: Colorectal liver metastasis; BMI: body mass index, SGOT: serum glutamic-oxsaloacetic transaminase; SGPT: serum glutamic-pyruvic transaminase; CBC: complete blood count; WBC: White Blood Cell; Hb: Hemhlobin; PLT: Platelet Count Test; SGOT: serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; SrCr: Serum Creatinine.

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