Correlation between High-Mobility Group Box 1 (HMGB1) and Carcinoembryonic Antigen CEA in Iraqi Colorectal Cancer Patients

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Abstract

Introduction: Cancer is a well-known public health problem, and it is a major cause of death worldwide. Colorectal Cancer (CRC), is the most common malignant cancer in the gastrointestinal tract and Carcinoembryonic antigen (CEA) is the most common diagnostic biomarker used in (CRC) and most of the gastric tumors which is used to identify/monitor or detect the type of tumoras it's used as a predictive and prognostic biomarker.High mobility group box protein-1 (HMGB1) proteins are one of the majorReceptors for Advanced Glycation End Products(RAGE)-ligands that contribute to inflammation consequences and an increased release of RAGE ligands, can play multiple roles in promoting the pathogenesis of (CRC), as it had been heavily implicated in the pathogenesis of (CRC).The research aims to detectthe possible relationship between CEA and HMGB1 in (CRC).

Methods: One hundred –forty unrelated participants, males and females, 90 of theme are CRC patients, besides 50 apparently healthy subjects, age & sex matching that of the patients, to serve as controls. The serum was obtained from each individual and stored frozen at -20 ℃ until the time of analysis, using specific ELISA kits for (HMGB1 and CEA), to determine their serum levels.

Results: Highly significant differences in serum levels (P=<0.0001) were obtained from both parameters, (85.33±2.86pg/ml)for CEA and (1.14±0.05ng/ml) for HMGB1 by the control group compared with (171.16±4.59 pg/ml) and (2.72±0.08ng/ml) for CEA and HMGB1respectively in patients' group.Also, a high sensitivity and specificity for both parameters had been detected, with a remarkable criterion accuracy (cutoff point) and positive correlation between those parameters in the current study.

Conclusion: Thecurrent study concludes that HMGB1 level shows similar sensitivity and specificity with a criterion accuracy (cutoff point) as forCEA and could be used for routine monitoring, diagnosis, and follow the prognosis for CRC as well as CEA does, with better diagnostic accuracy comparing with CEA, especially for early-stage CRC.

Recommendation: current study suggests using HMGB1 as a diagnostic parameter for CRC.

KeyWords: Cancer, CRC, CEA, HMGB1.

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Introduction

Cancer occurs whencells start to abnormally divide and grow without any control and become capable of invading other tissues through the blood and lymphatic system, through metastasis [1]. Cancer is a well-known public health problem, and it is a major cause of death worldwide[2].colorectal cancer (CRC) is a slowly developing cancer type that begins as a tumor or tissue having growth on the inner lining of the rectum or colon. If this abnormal growth, eventually becomes cancerous, it can form a tumor on the wall of the rectum or colon, and subsequently grow into blood vessels or lymph vessels, increasing the chance of metastasis to other sites in the body [3,4].

Worldwide,Colorectal Cancer (CRC), is the most common cancer in the gastrointestinal tract, and representing 13% of all malignant tumors, affecting men as women, in the same manner, in developed and undeveloped countries, and it is expected to overcome the mortality rate of heart diseases in the coming years [5,6]

In Iraq, (CRC) has increased in the last 10 years, and which become the leading cause of about 10% of cancer mortality. It becomes the second and third most common cancer in women and men respectively [7].

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Many risk factors have been contributed to (CRC) including diet with the strong relationship between (CRC) and high intake of red meat and processed meat [8], a Western diet rich in fat [9], cigarette smoking, and the use of tobacco in all forms [10,11], gastrointestinal inflammation

andhyperinsulinemia [12,13]. As well as family history has great relevance to the risk of CRC [14].

Carcinoembryonic antigen (CEA),a diagnostic biomarker which is used to identify/monitor or detect the type of tumor, its also used as a predictive biomarker (to predict the efficacy or response to different treatments or therapeutic interventions), and prognostic biomarker (to indicate the progress of disease and to estimate the risk of disease recurrence [15]. In (CRC), CEA has always been recommended as a reliable tumor marker by the National Comprehensive Cancer Network (NCCN) and the American Society of Clinical Oncologyto be used postoperatively and preoperatively for CRC surgery.[16]

Advanced Glycated End Products,heterogeneous group of moleculescollectively called advanced glycation end products (AGEs), which areproduced by glycation in cells or long-lived extracellular proteins[17]. RAGE (receptor for advanced glycation end-products) is a surface protein with a mass of 45–55 kDa, in the structure of which we can distinguish three fragments with different functions [18].The affinity of RAGE to AGEs is high, and the complexes between the ligand and the receptor are formed already within nanomolar concentrations [19].This particular receptor (RAGE) is a non-specific multi-ligand pattern receptor that induces the inflammatory responses by binding with multiple ligands. RAGE and its ligands are upregulated in diabetes, inflammation, and cancer. High mobility group box protein-1 (HMGB1) proteins are one of the major RAGE-ligands that contribute to these consequences and an increased release of RAGE-ligands during diabetic conditions can be a possible mechanism leading to diabetic complications and cancer [20].As a tumor-promoting agent, tumor cell-released (HMGB1) enhanced immunosuppressive cell recruitment, tumor angiogenesis, invasion, and metastasis. [21].HMGB1 plays multiple roles in promoting the pathogenesis of CRC, and may differentially regulate disease-related processes, depending on the redox status of the protein in CRC, HMGB1 has heavily implicated in the pathogenesis of CRC [22].

Aim of the Research

Study the relationship between serum levels of CEA and HMGB1 in Iraqi CRC patients.

Subjects and Methods

One hundred–forty unrelated participants, males and females, were recruited from the Oncology Hospital at Baghdad Medical City-Complex, Baghdad/ Iraq. during the period from April/ 2021 to February/ 2022, with an age range of (27-78 years) both (males and females). Ninety subjects were diagnosed to have CRC (60 patients with colon cancer and 30 patients with rectal cancer) by a specialized physician, first by CT scan, ultrasound scan, colonoscopy, and tumor markers supported by information recorded in the hospital by physical and clinical examination. In addition to fifty apparently healthy subjects, with age & sex matching that of the patients, to serve as controls (Table-1).

Table1. Gender and BMI distribution between patients and controls

Factor	Patients	controls	p-value
Gender	N=90	N=50	
male	52	27	0.66
female	38	23	
BMI			
less than 18.5	20	9	0.14
18.5- 24.9	32	24	
25-29.9	24	13	
30 or more	14	5	

N=number; BMI= Body Mass Index; P-value >0-05 non-significant.

Patients enrolled in this research were not diagnosed with other types of cancer, not previously on chemotherapy, did not have colorectal cancer surgery, not having chronic diseases like cardiovascular, diabetes mellitus, chronic kidney disease, and hypertension.

Blood Specimens had been collected, by taking 5-7 ml of venous blood from each patient and healthy control. Each sample has been separated into a serum-separating tube. The serum is then separated by centrifuging for 10 minutes at 5000 rounds per minute (rpm) and stored as aliquots in Eppendorf tubes to be kept frozen at -20 C° until the time of analysis, using specific ELISA kits for (HMGB1 and CEA), using a defined specific procedure provided by kit supplier [23,24].

Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). The receiver operating characteristic curve (ROC curve) was used to identify the validity of markers as an

indicator of the disease. The markers were compared according to the area under the curve. The correlation coefficient also was estimated. The analysis was submitted using MedCalc Software.P < 0.05 is considered statistically significant.

The reference for statistical analysis is SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016)".

SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

The sensitivity (a probability that the test will be positive when the infection is present) was calculated using the following formula:

Sensitivity (Sn)%=TP/(TP+FN) x 100

Where;TP= True positive test, FN=False negative test

The specificity (a probability that the test will be negative when the infection is absent) was calculated using the following formula:

Specificity (Sp)%= TN/(TN+FP) x 100

Where;TN= True negative test, FP= False-positive test.

The cutoff point was determined by using Youden Index. Also, the Chi-square test was applied to test for significant comparison between percentage and least significant difference –LSD test was used for significant comparison between means in this study.

Results

Serum levels of CEA were highly significantly elevated (P<0.0001)in CRC patients as well as serum level of HMGB1, which were highly significantly increased (P<0.0001) as shown in table-2. Furthermore, the sensitivity and specificity of test are shown in figure-1 and figure-2 respectively.

Table2. Serum Levels of CEA & HMGB1

Groups	CEApg/ml	HMGB1 ng/ml
Control	85.33±2.86	1.14±0.05
Patient	171.16±4.59	2.72±0.08
P-value	<0.0001	<0.0001

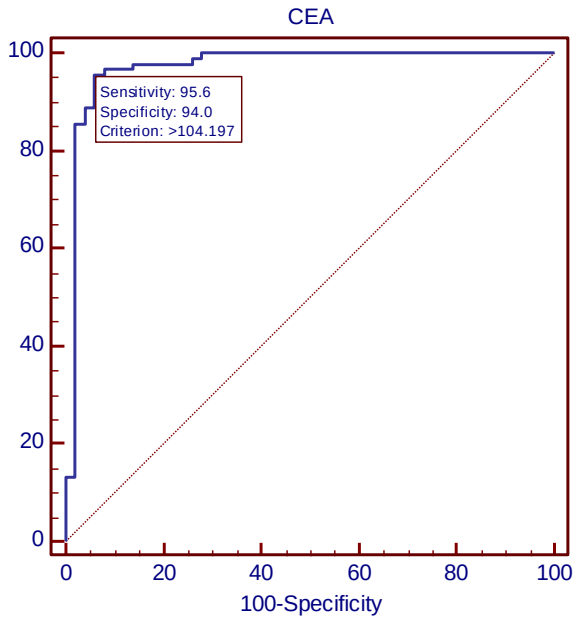

Figure1. Sensitivity and Specificity of CEA

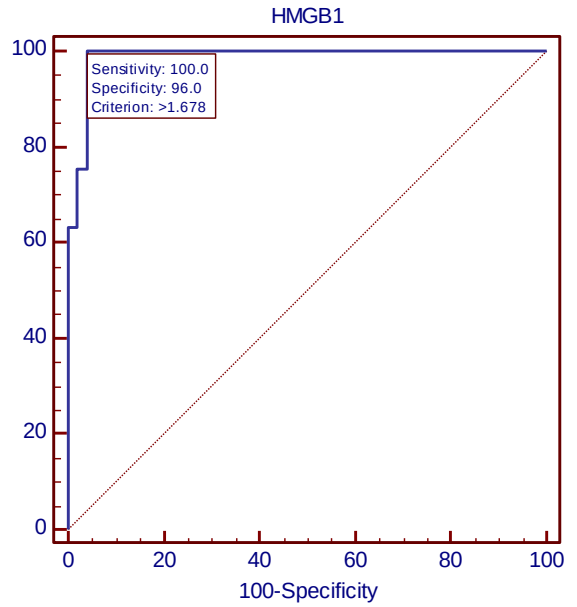

Figure2. Sensitivity and Specificity of HMGB1

Correlation Study

As shown in tables-3 and -4 and Figure-3, there is a correlation between the CEA and HMGB1 in the current study. Data indicates the presence of no significant differences between studied parameters, which means the possibility use of any of them as diagnostic marker for CRC using specific Criterion for each of these parameters above as shown in table-3and table-4. The current study also found appositive correlation between CEA and HMGB1 as shown in figure-3.

Table3. Sensitivity and Specificity of the Parameters in the Current Study

Variable	AUC	Criterion	SE [a]	95% CI [b]
CEA	0.972	> 104.197	0.0180	0.929 to 0.992
HMGB1	0.988	> 1.678	0.00893	0.953 to 0.999

Table4. Pairwise Comparison of ROC Curves Between the Parameters

CEA ~ HMGB1	
Significance level	P = 0.4113

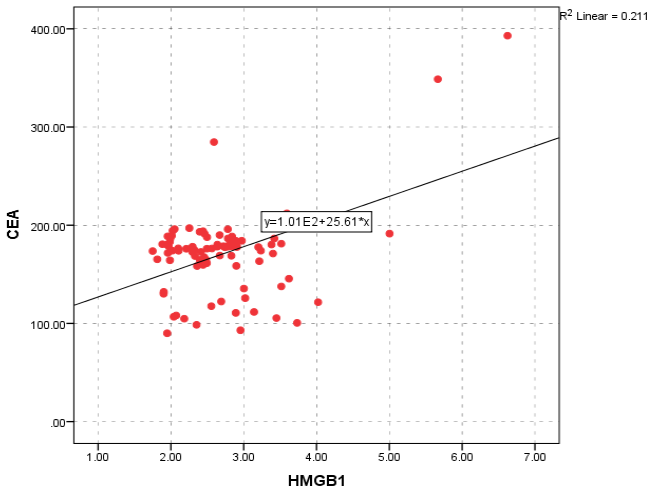

Figure3. Correlation Between CEA and HMGB1

Discussion

The above results for CEA were agreed with the results published by Leilani Lakemeyer *et.al.*[25] who indicate the use of CEA in the guidelines which recommended the use of this parameter for determining prognosis, surveillance after curative resection, and monitoring treatment of CRC. Rashmi Sreedhar *et.al.,*[26] also reported that CEAmay be used for staging the disease and planning the surgical intervention. Raised preoperative CEA at a certain level is associated with a poor prognosis.CEA levels are important indicators for recurrence in asymptomatic CRC patients and are cost-effective compared with radiology for detecting recurrence and metastases. On another hand,Hai Luo *et.al.,*[27] showreasonable specificity and sensitivity of CEA as shown in the current study.While, Martin Pesta *et.al.,*[28] suggest high specificity but low sensitivity for using CEA in the detection of recurrent disease.Han-Gil Kim*et.al.,*[29] suggest that CEA is significantly high preoperatively as suggested by the current study result.

Those results for HMGB1are agreed by Kim Jun Cheng *et.al.,*[30] which clarify the role of HMGB1 in the pathogenesis of CRC and indicate specifically that HMGB1 is heavily implicated in the pathogenesis of CRC, while Chuanping Yuan and Ling Yang [31] indicate not only the observably elevated HMGB1in CRC tissues, but also could serve as a treatment target for CRC patient, and their discoveries demonstrate that HMGB1 knockdown can inhibit inflammation.

Furthermore, Fei Qian *et.al.,*[32] indicate that the HMGB1-RAGE signaling plays an integral role in inflammation-driven carcinogenesis in CRC cells. While Sei-ichi Tanuma *et.al.,*[33] indicate that the suppressing of the HMGB1-RAGE axis provides an effective strategy for CRC treatment.

According toAli A Ghweil *et.al,*[34] HMGB1is used as a biomarker for many gastric tumors including CRC but he indicatesspecifically that CEA is significantly inferior to HMGB1 as a gastric cancer biomarker.

Meanwhile,He S, Cheng J, Sun L, *et al.*[35] found that HMGB1 overexpression plays an important role in tumor progression and metastasis in CRC, and therefore it may be considered a significant predictive factor.Huang *et.al.,*[36]proved that significant increases were found in the expression of the serum HMGB1 concentration in CRC patients. The expression and HMGB1 concentration were remarkably higher in the samples from CRC patients with distant metastasis, indicating that they are correlated with the clinical severity and prognosis of CRC.

A recent study by Nimet Yilmaz *et.al,*[37] reported that HMGB1 is a key factor involved in the growth, progression, angiogenesis, invasion, and metastasis of CRC. According to that, HMGB1 might be a promising biomarker in predicting clinical outcomes in CRC patients, especially with the diagnostic accuracy of serum HMGB1 which is markedly greater than that of carcinoembryonic antigen (CEA), which is the most commonly used serum marker in the early diagnosis.

Conclusion

Current study concludes a positive correlation between HMGB1 and CEA in Iraqi Patients and can be used as alternative biomarkers. Both show high accuracy and sensitivity, which makes HMGB1 reliable to be used as a potential diagnostic and prognosis monitoring biomarker in CRC, our recommendation is to establish more studies including HMGB1 as a biomarker for other types of

cancer.

Recommendation

The current study suggest using HMGB1 as diagnostic parameter for CRC.

Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

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