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CA72-4 as a promising prognostic and diagnostic biomarker in Iraqi patients with colorectal cancer

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ABSTRACT

Colorectal cancer (CRC), the second most fatal cancer and the 3rd most common cancer is expected to cause 0.9 million deaths globally in 2025. Carcinoembryonic antigen (CEA) is currently used in the follow-up of patients with colorectal cancer, and in this study, we are trying to find a better marker than CEA in following up on patients' health and knowing the effectiveness of the treatment used and as a diagnostic marker for colorectal cancer. To determine the significance of Cancer antigen 72-4 (CA72-4) as a prognosis predictor in patients with colorectal cancer, compare its prognostic validity to the CEA biomarker. this case-control study includes (150) participants, 100 patients (59 males and 41 females), and 50 healthy controls (26 males, 24 females). Blood samples were collected from all participants to measure the serum concentrations of CA72-4 and CEA using an enzyme-linked immunosorbent assay (ELISA). Between November 2020 and February 2021 in Baghdad, Iraq, this investigation was conducted at the oncology teaching hospital's gastrointestinal consulting clinic. There was a strong positive relation between CA242 and CEA (R = 0.953, p <0.001) and participants with colorectal cancer had considerably greater levels of CA72-4 than healthy controls (p <0.001). AUC was 0.944, sensitivity was 86%, specificity was 94%, and the cutoff value was 50 U/ml for the CA72-4. while AUC was 0.919, sensitivity was 91%, specificity was 80%, and the cutoff value was 5 ng/ml for the CEA.CA72-4 can serve as a potential prognostic and diagnostic biomarker for colorectal cancer.

KEYWORDS: CA72-4; CEA; colorectal cancer; maker; ELISA.

الخلاصة

سرطان القولون والمستقيم، وهو ثاني أكثر أنواع السرطانات فتكًا وثالث أكثر أنواع السرطان شيوعًا، ولقد حدث **0.9** مليون حالة وفاة على مستوى العالم في عام **2025**. مستضد سرطاني مضغي **((**CEA) ويستخدم حاليًا في متابعة مرضى سرطان القولون والمستقيم، ونحاول في هذه الدراسة إيجاد معلم أفضل من CEA في متابعة حالة المرضى ومعرفة فعالية العلاج المستخدم. لتحديد أهمية مستضد السرطان **((**CA72-4) كمؤشر للتنبؤ في مرضى سرطان القولون والمستقيم، قارن صحته التنبؤية بالمعلم الحيوية. CEA تشمل هذه الدراسة **150** مشاركًا ، **100** مريض (**59** من الذكور و **41** من الإناث) و **50** عنصر تحكم سليم (**26** ذكرًا و **24** أنثى). تم جمع عينات الدم من جميع المشاركين لقياس تركيزات مصل CA72-4 وCEA باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم. **((**ELISA) بين تشرين الثاني (نوفمبر) **2021** وشباط (فبراير) **2022** في بغداد، العراق، أجريت هذه الدراسة في العيادة الاستشارية للجهاز الهضمي في مستشفى الأورام التعليمي. كان لدى المرضى المصابين بسرطان القولون والمستقيم مستويات أعلى بكثير من CA72-4 من المجموعة الضابطة (01.**00>**p) مع وجود ارتباط إيجابي كبير بين CA242 و CEA، و **((**R = 0.953) كشفت دراسة منحنى CA72-4 ROC أن AUC تبلغ **0.944**، وحساسية **86**٪، وخصوصية **94**٪، وقيمة قطع **50** U/ml بينما كشفت دراسة منحنى CEA ROC أن AUC 0.919، حساسية **91**٪، وخصوصية **80**٪، وقيمة قطع **5** نانو غرام / مل. يمكن أن يكون CA72-4 بمثابة علامة بيولوجية تنبؤية محتملة لسرطان القولون والمستقيم كما يمكن استخدامه في تشخيص المرض.

INTRODUCTION

Colorectal cancer (CRC) classified as the second-deadliest cancer in the world and the 3rd most frequent cancer [1]. By 2030, it is predicted that there would be 2.2 million additional cases of CRC and 1.1 million mortalities [2]. According to the American Cancer Society, one in every 21 men and one in every 23 women in the United States will develop advanced CRC at some point in their lives [3]. In Iraq, the prevalence of CRC is moderate, though it has been steadily increasing over time [4]. The extent of the tumor at the point of diagnosis is

known to affect the prognosis for colorectal cancer. Therefore, if it is detected and managed at an early stage, colorectal cancer will be long term survival [5].

CEA glycoproteins found in growing fetus in various cell types, linked to cancers. The term "carcinoembryonic" refers to the fact That new cells of tumors produce CEA [6], so CEA test has been extensively acknowledged as an additional test for prognosis prediction and as a management tool for cancer patients [7].

CA 72-4 is a glycoprotein found on the surface of many cancer cells, has been found to be elevated in a number of human adenocarcinomas, it can be detected in individuals with stomach, ovarian, pancreatic, breast, rectum and colon cancers. Previous studies have demonstrated elevated CA72-4 levels in cancer patients, which also increased as the disease progressed. However, its potential use as a diagnostic marker in suspected individuals is still being studied [8]. Aim of study: To determine the significance of Cancer antigen 72-4 (CA72-4) as a prognosis and diagnostic marker for patients with colorectal cancer, and compare its prognostic and diagnostic validity to the Carcinoembryonic antigen (CEA) biomarker.

MATERIALS AND METHODS

The ethics committee of the Baghdad University College of Medicine as well as the Ministry of Health's Training and Development Section in Baghdad /Iraq approved the study. patients gave their consent before the samples were taken, and being given both written and verbal information regarding the study's importance. This case control study, which was conducted between November 2021 and February 2022, is a prospective study. The CRC group of patients were diagnosed by oncology consultant after a clinical examination, laboratory tests, a CT or MR radiographic inspection, and a histopathological diagnosis were then used to confirm the diagnosis. Patients group included 59 males and 41 female age ranging from (26 to 75) years old (mean± SD, 50.58± 11.38 years). The patient CRC group involve 100 patients with CRC of the rectum cancer (n=16) and colon cancer (n=84). The control group comprised of 50 healthy participants (ages 34 to 68), mean± SD (49.9 ± 8.45) years, 26 men and 24 women who were free of any serious infections or gastrointestinal problems. The following criteria were used for inclusion: complete information on preoperative serum oncologic biomarkers, complete clinical histopathologic assessment. The patient group was split into three subgroups: those receiving only chemotherapy (subgroup 40); those receiving both chemotherapy and immunotherapy (subgroup 32); and those receiving follow-up treatment (subgroup 28); and individuals who appeared to be in good health (50). The Enzyme Linked Immunosorbent Assay (ELISA) technique was utilized by Teaching Laboratories/Medical City, 5ml of intravenous blood were drawn from every colorectal cancer patient and control participants. with each sample of blood (5 ml), we used plain tubes and let the blood coagulate for 10 to 20 minutes at room temperature, Centrifuge for 10 min between 2000 and 3000 RPM, carefully take the supernatants, Storage: The kit is stored between 2 and 8 degrees Celsius.

Statistical analysis

The statistical analysis was performed using the SPSS 26 statistical software package, basic results are expressed as a mean ± standard deviation, Standard descriptive statistics, tabulation of categorical categories, and histograms of numerical variables were utilized to characterize the data. The nonparametric Mann-Whitney analysis was used to measure the median difference between the two groups. The ANOVA test was used to determine statistical differences between the groups, and student t-test findings were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

The case control study has a sample size of 150 participants, the age 50.58 ± 11.38 years for the patient group and the control group 49.9 ± 8.45 years, the age of the patients is divided into 2 groups: (age ≥ 50) includes (58) individuals, and (age<50) includes (42) subjects. The patients' age range is (26 to 75), Compared to (50) a control healthy individuals age range between (34 and 68), there are (59) male and (41) female colorectal cancer patients in the patient group, while the control group include (26) male (52 %) and (24) female (48%), in total of both groups the male consists (56.6%) and female (43.4%).

Patients group include (19%) of subjects have family history of cancer, while (81%) of the cases do not have history. The patients' groups were (21) smoking with (79) of the patients were nonsmoking. The mean concentration of serum CEA in total patients (19.24 ng/ml) and patent's

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groups (on chemotherapy only, on chemotherapy and immunotherapy, on follow up) are significant ($p<0.001$) higher (19.88, 19.79, and 16.99 ng/ml, respectively) than control group (3.45 ng/ml). Furthermore, the mean concentration in subgroups of the patients was, with no statistically significant, all explain in Table 1.

Table 1. Serum concentration of (CEA) in study groups [9].

Group	M±SD (ng/ml)	Chemo therapy	Follow up	Control
Chemo/Immunotherapy	19.69±6.57	0.95	0.1	<0.001
Chemotherapy	19.78±7.62		0.06	<0.001
Follow up	16.89±6.89			<0.001
Control	3.35±2.21			
Total Patients	19.14±8.28			<0.001

The mean serum concentration of CA 72-4 was higher in total patients (73.07 U/ml) than the mean serum level of CA 72-4 in control group (13.75 U/ml) with statistical significance among patients and control groups ($p > 0.01$). The mean serum concentration of CA72-4 in subgroups of patients (chemotherapy only, chemo/immunotherapy, follow up) was also significantly higher compared to control (69.15, 71.39, and 67.99 U/ml, respectively), with no statistical significance when compere between these subgroups, see in Table 2.

Table 2. Serum level of (CA 72-4) in between study groups.

Group	M±SD (U/ml)	Chemo therapy	Follow up	Control
Chemo/Immunotherapy	71.93±16.24	0.41	0.29	<0.001
Chemotherapy	69.15±15.54		0.74	<0.001
Follow up	67.99±17.55			<0.001
Control	13.75±8.54			
Total Patients	73.07±21.0			<0.001

The correlation of CEA with CA72-4 was positive ($R=0.953$, $P > 0.05$), Figure 1. The CEA ROC curves under curve area (0.91) indicates a significant association between the prognosis value of the disease and the prognostic markers, as well as its sensitivity (91%) and specificity (80%) with a cutoff of 5 ng/ml. and for the cutoff value for the (CA72-4) marker was (50 U/ml), and its sensitivity (86%), specificity (94%), and under the curve area (0.94) are all highly correlated with the disease's prognosis. the sensitivity is higher in the CEA while the specificity was higher in the CA 72-4. The area under curve was higher in the CA 72 than CEA. See Table 3.

Other study [10] is showing that individuals of different ages can get colorectal cancer. In recent years, most cases of colorectal cancer occur in patients group at age ≥ 50 (58%), while early cases of the disease rise in persons at age < 50 (42%). in many regions of the world, young adults are now more common among older adults to develop colorectal cancer, this is agreed with other study [11].

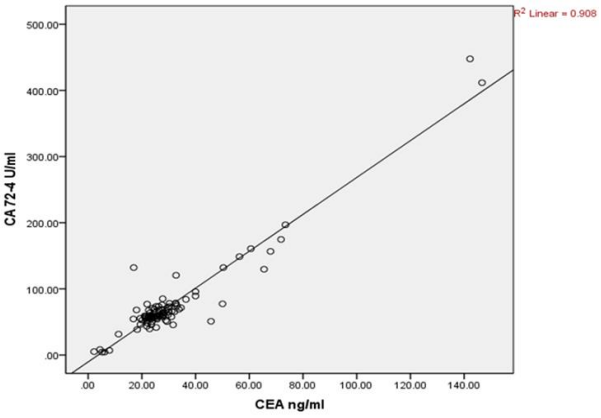

Figure 1. Correlation between CEA and CA-242.

Table 3. ROC curve for makers.

Makers	Cut-of	Sensitivity	Specificity
CEA	5 ng/ml	91%	80%
CA 72-4	50 U/ml	86%	94%

The patients in this study group ranged in age from (26 to 75), which is consistent with previous findings that men are more likely than women to develop CRC. Furthermore, the study backs up another study [12], which found that men are more likely than women to smoke, drink more alcohol, and consume a lot of red meat in their diet. These findings back up another study [13], which found that males were more impacted than females, and also agree with another Iraqi study [14]. In this study, in patients group non-smokers comprised (79%) of the patients and smokers (21%), causing cigarette consumption one of the probabilities of developing colorectal cancer. This is agreed with other study [15], that state Smoking was linked to mutation-positive colorectal cancer, supporting the hypothesis that epigenetic changes may play a functional role in the progression of colorectal cancer as a result of smoking. The majority of colorectal cancers patients are found in who have no family history of cancer, which may be because of various risk factors that lead to the improvement of cancer. Patients with a family history of cancer composed 19 % of the patient group, compared to those with no family history, who composed (71%) of the patient group, this is agree with other study [16], that state About 35% of those who have the disease have a family history of colorectal cancer (CRC) carried on by genetic predispositions, environmental exposures, or both. Only 5 to 10 percent of CRC cases are linked to specific families with a history of cancer and high- or moderate-incidence genetic variants that cause CRC. The intestinal tissue of humans from the embryonic stage to the fetus expresses a group of tightly connected glycoproteins known as carcinoembryonic antigen (CEA). In adults, colon mucosal cells primarily produce CEA, with a minor amount coming from other cells. There is only a small amount of spread in the blood. In blood, it has a half-life of two days. Being a highly expressed embryonic cancer antigen in colorectal cancer, CEA is used as a diagnostic marker in clinical practice [17].

The colorectal cancer patient group means serum CEA level in this study was 19.20ng/ml, which is high significantly statistically ($p< 0.001$) from the control group's level of 3.40ng/ml. this agree with other study [18], shows that patients with colorectal cancer have a high level of CEA.

In this study, the patients were divided into the following categories according to the type of therapy they received (chemotherapy only, chemo/immunotherapy), and in these subgroups, the mean CEA level was (19.88 ng/ml and 19.79 ng/ml) respectively. These high levels may be related to the short time received treatments; the CEA levels should return to normal within 4 to 6 weeks following successful surgical excision of colorectal cancer and treatment. agree with other study [19], a brief increase (surge phenomenon or flare) was connected to a therapeutic advantage in patients receiving first-line chemotherapy for metastatic colorectal cancer. which agree with other study [20]. The CEA test is an effective tool for assessing the prognosis of CRC patients and has a good specificity and sensitivity for CRC screening, in recent years, biomarkers have demonstrated their potential to aid in the establishment of diagnosis and the assessment of treatment effects and prognosis [21].

CA72-4 was discovered to be a new antigen that was recognized by mouse antibodies generated by animals vaccinated with membrane-enriched portions of human metastatic cancer cells. The monoclonal antibody B72.3, a murine antibody produced against human cancer metastasis, recognized this 48-kDa mucin-like glycoprotein complex. This antigen was already detected in human colorectal cancer [22].

In this study, the colorectal cancer patients' mean serum levels of CA 72-4 were (73.07 U/ml), a significant statistical difference ($p< 0.05$) with the control groups' (13.75 U/ml), this is agreed with other study [23], which state that CA72-4 levels of serum can also be used to predictable outcome after chemotherapy and to evaluate for chemotherapy resistance. CA72-4 levels in the serum dropped by more than 70%, indicating a good pathological response to therapy and indicating that individuals with declining CA72-4 had a good prognosis. In this study, notice that the subgroups of patients (chemotherapy only, chemo/immunotherapy) mean serum concentration was (69.15 U/ml), (71.39 U/ml) respectively, while the mean serum concentration of follow up subgroup was (67.99 U/ml), which consider high level may that due to spread of metastases, or maybe there is chemotherapy resistance, this is agree with other study [24], stated that CA724 has the potential to predict lymph node cancer spread and pathological stage, were independent prognostic criteria following treatment. There is no significant statistical difference between the subgroups. The

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CA 72-4 cutoff value for patients was 50 U/ml, as according ROC analysis, with a sensitivity of 86%, specificity of 94%, and an area under curve of (0.94). and CEA as a traditional marker had a strong correlation with CA72-4, The CEA cutoff value was 5ng/ml, with sensitivity (91%) and the specificity (80%).

CONCLUSIONS

The aim of the research was to measure the levels of the CA72-4 maker in patients with colorectal cancer and the control group, to demonstrate the possibility of using the marker in the early detection of colorectal cancer, as well as to demonstrate the relationship between the marker and carcinoembryonic antigen (CEA) that currently used to detect the colorectal cancer and to compare the sensitivity and specificity of each of them.

The study concluded that the levels of the parameter was high in colorectal cancer patients compared to the control group, with significant differences and the possibility of using the marker in the diagnosis of colorectal cancer.

It can also be used in evaluating the cases of patients with colorectal cancer to follow up the therapeutic efficacy in different treatment stages, reaching the point of recovery, because the marker is linked to the presence of cancer and not found in normal tissue.

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