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Immunological and physiological profiles in colorectal cancer patients – a comparative analysis based on metastatic status

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ABSTRACT

Introduction and aim. Colorectal cancer is the third most prevalent cancer in the world. However, the biochemical factors associated with metastatic compared with non-metastatic colorectal cancer remain incompletely characterized, particularly in regional populations. This study aimed to investigate the associations of leptin, carcinoembryonic antigen (CEA), liver enzymes, total protein, and total bilirubin with metastatic status in patients with colorectal cancer.

Material and methods. Leptin, CEA, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and total bilirubin levels were measured in 120 colorectal cancer patients, divided into metastatic and non-metastatic groups. Multivariable binary logistic regression and receiver operating characteristic curve (ROC) analyses were used to evaluate independent associations with metastatic status and the discriminatory performance of the investigated biomarkers.

Results. In the unadjusted between-group analysis, patients had significantly different levels of leptin and CEA between metastatic and non-metastatic disease ($p<0.007$). Significantly higher mean values of AST, ALT, ALP and total bilirubin were also observed in the metastatic group as compared to the non-metastatic

group ($p<0.007$). After multivariable adjustment, CEA, AST, ALT, ALP, and total bilirubin remained independently associated with metastatic status, whereas leptin and total protein were not independently associated with metastatic status. Furthermore, leptin did not demonstrate significant discriminatory performance in ROC analysis.

Conclusion. CEA, AST, ALT, ALP, and total bilirubin were independently associated with metastatic status and demonstrated discriminatory potential in distinguishing metastatic from non-metastatic colorectal cancer. These findings support their potential value as markers associated with metastatic disease; however, further validation in independent cohorts is required before their clinical diagnostic utility can be established.

Keywords. bilirubin, carcinoembryonic antigen, colorectal cancer, leptin, metastatic

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Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide, and it is much more common in middle- and high-income countries, with a 8 fold regional difference in incidence frequency.[1] This rise may be attributed to factors that have been recognized as being risky to health, including alcohol consumption, smoking, obesity, lack of activity and nutrition.[2,3] In summary, CRC is a substantial public health problem in terms of morbidity, mortality and the cost of health services, which is increasing at a rapid rate,[4] with up to 20% of patients already having metastases when they are first diagnosed, and up to 50% of those with localized disease having developed metastases at some point during their course of illness.[5] Once metastases appear, the disease becomes highly lethal, with a 5-year survival rate of only about 14%.[6] Appropriately managing these patients remains a challenging clinical problem.[5,7]

Leptin is a peptide hormone mainly secreted by adipose tissue. It performs its typical roles to control food intake, appetite, energy expenditure, energy balance, immune responses, and reproduction.[8] As fatty acids are essential for the growth of cancer cells, it is not surprising to suspect that adipocytes play a role in the growth and metastasis of these cells.[9] Leptin has a well-established role in the promotion of various cancers, including both adenocarcinomas and squamous cell carcinomas.[10] Evidence also suggests leptin plays roles in immune function, tumor invasion, and metastasis. [11]

The most widely used serum biomarker for CRC is carcinoembryonic antigen (CEA), which is a protein that is excreted during fetal development and by some cancer cells.[12,13] CEA is normally low in healthy people but can be elevated in CRC patients. But high levels of CEA can also be found in other diseases, such as inflammation of the colon. Nevertheless, it is still useful in monitoring for CRC recurrence or disease progression.[14]

Aminotransferases specifically aspartate transaminase (AST) and alanine aminotransferase (ALT) are common blood-based markers of liver injury.[15,16] Several retrospective studies have demonstrated that a high AST and ALT levels is associated with cancer prognosis in several malignancies.[15,17]

Bilirubin, an endogenous antioxidant, is routinely used to diagnose liver diseases. For a long time, it was viewed simply as a harmful waste product. However, more recent research has uncovered a connection between bilirubin levels and cancer.[18] Another recent study noted that bilirubin possesses antioxidant and anti-tumor properties.[19] A level of moderately elevated bilirubin is actually correlated with a lower risk of cancer and a better prognosis, while very high levels of bilirubin are associated with a greater cancer risk.[20]

Aim

The changes in the expression of the different biomarkers in CRC have been studied worldwide, although there are limited data available on changes in the expression of these biomarkers in the region according to the metastatic status. To fill this gap, this study aimed to evaluate and compare leptin, carcinoembryonic antigen (CEA), and routine biochemical and liver-related markers in colorectal cancer patients with and without metastasis, to assess their independent associations with metastatic status after adjustment for potential confounding factors, and to evaluate their discriminatory performance in distinguishing metastatic from non-metastatic disease. The novelty of the present study lies in the simultaneous evaluation of these markers within the same regional cohort using complementary unadjusted between-group comparisons, multivariable analysis, and ROC analysis. The results could help with the understanding of metastatic CRC and could provide as a foundation for additional validation studies.

Material and methods

Patient selection

A prospective observational comparative study was conducted in the Oncology Teaching Hospital from January 2025 to February 2026 involving 120 patients with CRC. Patients were categorized into two equal groups: 60 patients with metastatic disease and 60 patients without metastatic disease. Patients were recruited during treatment sessions at the Hospital and enrolled based on specific eligibility criteria. Primary colorectal cancer was diagnosed by pathological examination of biopsy or surgical specimens. The metastatic status was established at enrollment and confirmed using baseline imaging modalities, including contrast-enhanced computed tomography (CT) scans and magnetic resonance imaging (MRI), in alignment with institutional oncology board reviews.

The sample size was calculated using G*Power software (version 3.1). Based on an independent samples t-test, with a significance level (α) of 0.05 and a statistical power (1-β) of 80% to detect a medium-to-large effect size (Cohen's d=0.52), the minimum required sample size was determined to be 60 patients per group. Thus, 120 patients were recruited for this study. All data were gathered adhering to the ethical principles of

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the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment in the study. Patient specific information was protected and not disclosed. Record numbers were also coded for the purposes of confidentiality. Finally, the study received the approval of the Ethics Committee of Mustansiriyah University (approval no. BCSMU/1024/0050Z).

Inclusion criteria

Inclusion criteria were as follows: Primary colorectal cancer from any part of the colon. Confirmed metastatic disease that is limited to intra-abdominal sites or limited (resect able/non-extensive) liver metastases. All patients must have undergone surgical resection of the primary tumor site. Patients who receive first-line systemic oncological treatment. No history of chronic systemic diseases in the past and non-alcoholic history. A performance status of the ECOG (Eastern Cooperative Oncology Group) scale 0 or 1.

Exclusion criteria

Patients were excluded from the study if they had any of the following: Any other histopathologic type of cancer except primary colorectal cancer. Patients receiving radiotherapy and/or targeted therapy (only standard first-line chemotherapy regimens were included). Patients who were not treated with surgical resection for their primary CRC.

Blood samples

Peripheral blood samples were obtained following surgery and prior to the first treatment with first line drugs, which would cause confounding impacts on the biomarkers studied. The researchers took blood samples from veins from 120 colorectal cancer patients. These samples were placed inside the tubes that contain gel and kept undisturbed at room temperature for 30 minutes for the blood to coagulate. After that, the serum was separated by a centrifugation of 2000×g for 10 minutes at 4°C. This serum was then aliquoted into smaller Eppendorf tubes and stored in the freezer at -20°C for further analysis. All specimens were given a distinct specimen number.

Calculation of body mass index (BMI)

BMI is calculated as body weight (kg) divided by the square of the height (m) raised to the power of 2 (BMI=body weight (kg)/height (m)x height (m)).

Serum marker analysis

For Leptin and Carcinoembryonic antigen (CEA), a 2 mL serum volume was used, enzyme linked immunosorbent assay (ELISA) kits for humans based on the instructions for use provided by the

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commercial supplier for leptin (cat. no. RE2739H), and CEA (cat. no. RE2768H) from Reed Biotech Ltd. According to the manufacturer's specifications, the analytical sensitivity assay of leptin was 9.38 pg/mL, with a detection range of 15.63–1000 pg/mL, while for CEA, the analytical sensitivity was 2.25 pg/mL, with a detection range of 15.63-1000 pg/mL. Intra-assay and inter-assay coefficients of variation (CV) were determined by the manufacturer to be ≤10% to be sure of precision and reproducibility. All calibration procedures were carefully conducted before sample analysis with the manufacturer's multi-level calibrators, and a standard curve was built. Each sample was analyzed twice and the mean value of each sample was used in subsequent analysis. In addition, 3 ml of the remaining serum was used for the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and total bilirubin levels using Cobas® (Roche Diagnostics).

Statistical analysis

All statistical analysis were performed using GraphPad Prism version 9.2 (GraphPad Software, LLC, San Diego, CA, USA). The normality of continuous variables was assessed using the Shapiro–Wilk test, and all analyzed variables demonstrated a normal distribution (P>0.05). Homogeneity of variance was evaluated using Levene's test. Such data are presented as (mean±standard deviation). Categorical data were compared using the Chi-square test with 95% Cis and shown as counts (N) alongside their corresponding percentages (%). To compare group means, the student's t-test was applied, with reporting mean differences with 95% confidence intervals (95% CI) and Cohen's d effect sizes. While differences in categorical counts were assessed using Fisher's exact test. Separate Multivariable binary logistic regression analysis was performed to assess whether each marker was independently associated with metastatic status after adjustment for potential confounding factors. Logistic regression models were constructed, with metastatic status as the dependent variable. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) and corresponding P-values were reported. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory performance of the studied markers in distinguishing metastatic from non-metastatic patients. The area under the ROC curve (AUC) with 95% confidence intervals was calculated for each marker. The optimal cut-off value was determined using Youden's index, and the corresponding sensitivity and specificity were calculated. To account for the inflation of the Type I error rate arising from multiple comparisons involving the seven investigated biomarkers, the Bonferroni correction was applied. The significance threshold was set at $\alpha=0.007$ (0.05/7). There were no missing data for the variables included in the statistical analyses. P-values below this adjusted threshold were considered statistically significant, whereas p-values ≥0.007 were considered statistically non-significant.

Results

Baseline demographic and clinical characteristics of the study groups are summarized in Table 1. The mean age was 55.566±10.002 years in the non-metastatic group and 50.366±13.046 years in the metastatic group;

although the unadjusted p-value was 0.015, this difference did not reach the prespecified Bonferroni-adjusted significance threshold (α=0.007). There were also no statistically significant differences between the two groups in sex distribution (p=0.270), histological grade (p=0.011), or BMI (p=0.550) after Bonferroni correction.

Table 1. Baseline characteristics across study groups*

Character	Non-metastatic group (n=60)	Metastatic group (n=60)	p
Age (Mean± SD)	50.366±13.046	55.566±10.002	0.015 [1]
Sex, n (%)			0.270 [2]
Male	36 (60%)	30 (50%)	
Female	24 (40%)	30 (50%)	
Stage, n (%)			0.0001[#2]
Stage I	0 (0%)	0 (0%)	
Stage II	32 (53.3%)	0 (0%)	
Stage III	28 (43.3%)	0 (0%)	
Stage IV	0 (0%)	60 (100%)	
Grade, n (%)			0.011[2]
G1	16 (26.6%)	4 (6.7%)	
G2	37 (61.7%)	45 (75%)	
G3	7 (11.7%)	11 (18.3%)	
BMI (Mean±SD)	27.227±3.048	27.672±4.882	0.550 [1]

* Data are presented as mean±standard deviation (SD) for continuous variables and as number (N) and percentage (%) for categorical variables, [1] – p-value was calculated using the unpaired Student's t-test for the comparison across the study groups, [2] – p-value was calculated using the Chi-square test for the comparison across the study groups, [#] – statistically significant after Bonferroni correction (p<0.007), n – number of patients in each group, the significance threshold was adjusted from 0.05 to 0.007 using Bonferroni correction

The results of the biomarker analysis (Table 2) showed that the serum levels of leptin and CEA were significantly higher in the metastatic group, compared to the non-metastatic group. Leptin levels were

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higher in metastatic patients (378.55±186.73 pg/mL) than in non-metastatic patients (281.04±208.10 pg/mL), with a mean difference of 97.51 pg/mL (95% CI: 26.02–168.98), Cohen's d=0.49, p=0.006. Similarly, CEA levels were significantly higher in the metastatic group (176.39±92.25 pg/mL) than in the non-metastatic group (90.17±74.20 pg/mL), with a mean difference of 86.22 pg/mL (95% CI: 55.95–116.49), Cohen's d=1.03, p<0.001. Liver related enzymes were also found to be significantly higher in the metastatic group compared to non-metastatic group. AST levels were 38.83±10.21 U/L in the metastatic group compared with 29.70±7.01 U/L in the non-metastatic group, corresponding to a mean difference of 9.13 U/L (95% CI: 5.96–12.30), Cohen's d=1.04, p<0.001. ALT levels were also higher in the metastatic group (45.63±21.43 U/L vs. 27.16±7.58 U/L), with a mean difference of 18.47 U/L (95% CI: 12.66–24.28), Cohen's d=1.15, p<0.001. Similarly, ALP levels were higher in metastatic patients (131.99±40.95 U/L) than in non-metastatic patients (96.64±30.05 U/L), with a mean difference of 35.35 U/L (95% CI: 22.36–48.34), Cohen's d=0.98, p<0.001. Total bilirubin was also significantly higher in the metastatic group (0.862±0.778 mg/dL) than in the non-metastatic group (0.431±0.140 mg/dL), with a mean difference of 0.43 mg/dL (95% CI: 0.23–0.63), Cohen's d=0.78, p<0.001. In contrast, total protein levels were lower in the metastatic group (56.11±18.49 g/L) than in the non-metastatic group (64.35±17.19 g/L), with a mean difference of −8.24 g/L (95% CI: −14.69 to −1.79), Cohen's d=0.46, p=0.012 this was however not a significant difference as defined in the Bonferroni adjusted α value (0.007).

Table 2. Comparison of clinical markers across study groups*

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* Data are expressed as mean± SD, differences between the two groups were analyzed using the unpaired Student's t-test, n – number of patients in each group, the significance threshold was adjusted from 0.05 to 0.007 using Bonferroni correction, # – statistically significant after Bonferroni correction (p<0.007)

Parameter (unit)	Non-metastatic group (n=60)	Metastatic group (n=60)	p	Effect Size (Cohen's d)	CI 95%
Leptin (pg/mL)	281.04±208.10	378.55±186.73	0.006#	0.49	[+26.02 to +168.98]
CEA (pg/mL)	90.17±74.20	176.39±92.25	<0.001#	1.03	[+55.95 to +116.49]
AST (U/l)	29.70±7.01	38.83±10.21	<0.001#	1.04	[+5.96 to +12.30]
ALT (U/l)	27.16±7.58	45.63±21.43	<0.001#	1.15	[+12.66 to +24.28]
ALP (U/l)	96.64±30.05	131.99±40.95	<0.001#	0.98	[+22.36 to +48.34]
Total protein (g/L)	64.35±17.19	56.11±18.49	0.012	0.46	[−14.69 to −1.79]
Total bilirubin (mg/dL)	0.43±0.14	0.86±0.77	<0.001#	0.78	[+0.23 to +0.63]

Multivariable logistic regression analysis showed that CEA, AST, ALT, ALP and total bilirubin are still independently associated with metastatic disease after adjusting for age, BMI, sex and tumor grade (Table 3). Metastatic disease was also strongly linked to CEA with an adjusted OR of 1.018 (95% CI: 1.011–1.025, p<0.001). Similarly, AST (adjusted OR=1.127, 95% CI: 1.065–1.191, p<0.001), ALT (adjusted OR=1.096, 95% CI: 1.056–1.138, p<0.001), and ALP (adjusted OR=1.035, 95% CI: 1.020–1.051, p<0.001) showed significant positive associations with metastatic disease. Additionally, metastatic disease was associated with total bilirubin (adjusted OR=11.413, 95% CI: 2.563–50.818, p=0.001). In contrast, leptin was not independently associated with metastatic disease after multivariable adjustment (adjusted OR=1.002, 95% CI: 0.999–1.004, p=0.128), nor was total protein (adjusted OR=0.980, 95% CI: 0.958–1.002, p=0.080).

Table 3. Multivariable logistic regression analysis of biomarkers associated with metastatic status*

Biomarker	Adjusted OR	95% CI	p-value

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Leptin (pg/mL)	1.002	0.999–1.004	0.128
CEA (pg/mL)	1.018	1.011–1.025	<0.001
AST (U/L)	1.127	1.065–1.191	<0.001
ALT (U/L)	1.096	1.056–1.138	<0.001
ALP (U/L)	1.035	1.020–1.051	<0.001
Total protein (g/L)	0.980	0.958–1.002	0.080
Total bilirubin (mg/dL)	11.413	2.563–50.818	0.001

* the Bonferroni-adjusted significance threshold for the seven biomarkers was α=0.007

ROC curve analysis was used to further assess the discriminatory ability of the biomarkers studied to distinguish patients with established metastasis from those with non-metastatic disease. The discriminatory performance of the biomarkers was different as evidenced in Table 4. CEA showed the highest AUC (0.802, 95% CI: 0.717–0.888), followed by ALT (AUC=0.771, 95% CI: 0.683–0.858), ALP (AUC=0.749, 95% CI: 0.660–0.839), and AST (AUC=0.737, 95% CI: 0.647–0.826). CEA, ALT, ALP, and AST had statistically significant discriminatory performance after Bonferroni correction (α=0.007) (p<0.001). Total bilirubin (AUC=0.627, p=0.013), total protein (AUC=0.618, p=0.022) and leptin (AUC=0.582, p=0.117) did not meet the Bonferroni adjusted p value.

Table 4. discriminatory performance of the studied biomarkers for discrimination between metastatic and non-metastatic groups*

Biomarker	**AUC (95% CI)**	**p**	**Optimal cut-off**	**Sensitivity (%)**	**Specificity (%)**
Leptin (pg/mL)	0.582 (0.480–0.685)	0.117	554.21	26.67	90.00
CEA (pg/mL)	0.802 (0.717–0.888)	<0.001	155.45	66.67	96.67
AST (U/L)	0.737 (0.647–0.826)	<0.001	41.40	50.00	93.33
ALT (U/L)	0.771 (0.683–0.858)	<0.001	43.98	63.33	93.33
ALP (U/L)	0.749 (0.660–0.839)	<0.001	131.78	63.33	86.67
Total protein (g/L)	0.618 (0.517–0.718)	0.022	56.00	50.00	73.33
Total bilirubin (mg/dL)	0.627 (0.527–0.726)	0.013	1.15	26.67	100.00

* Statistical significance was assessed using a Bonferroni-adjusted threshold of α=0.007 (0.05/7) to account for multiple comparisons, optimal cut-off values were determined using Youden's index

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Figure 1 illustrates the ROC of the studied biomarkers for discriminating between metastatic and non-metastatic patients. The ROC curves demonstrated variable discriminatory performance among the studied biomarkers, with CEA showing the highest AUC, followed by ALT, ALP, and AST, whereas leptin showed the lowest discriminatory performance.

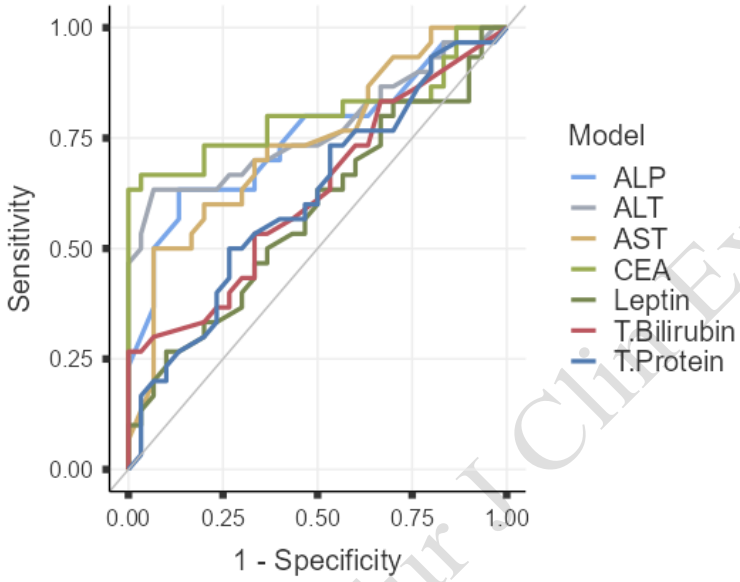

Fig. 1. ROC of the studied biomarkers for discrimination between metastatic and non-metastatic patients

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Discussion

CRC is the third most prevalent cancer, and the second leading cause of cancer-related death worldwide. Differences in incidence and mortality rates, time trends, and burden of CRC were reported by countries and regions.[21] These disparities most likely occur due to differences in exposure to lifestyle and environmental factors related to CRC.[1] Although much research has been conducted with respect to CRC, evidence for biomarkers specifically linked to metastatic status is still limited in some regional populations. The present study aims to fill this gap and examines selected biomarkers in metastatic and non-metastatic CRC patients.

There are many factors affecting progression of colorectal cancer, its initiation, promotion and progression. One of these factors is adipose tissue.[22] Among the various adipokines, leptin is the best known. Serving as a hormone.[23] Leptin has been shown to be one of the important factors promoting the proliferation and migration of tumor cells.[24] Leptin has been shown to stimulate the growth of cells and inhibit cell death in colon cancer.[24] Furthermore, several laboratory investigations have shown that leptin acts as a growth factor promoting cell proliferation and blood vessel growth and also inhibiting apoptosis, thereby supporting tumor development across different cancer cell lines.[25,26]

Our study showed higher mean leptin levels in patients with metastatic CRC than in those without metastasis (378.55±186.73 vs. 281.04±208.10 pg/mL; p=0.006) (Table 2). This association was not significant after adjustment for age, BMI, sex and tumor grade in the multivariable analysis (P=0.128) (Table 3).

The findings of these studies are in agreement with previous studies. For instance, in a study by Tutino and others,[27] levels of leptin and its receptor led the researchers to propose a link between leptin and both the progression and aggressive nature of colorectal cancer. Thus, the long-lasting high expression seen in colorectal cancers with liver metastasis may be due to the poor prognosis that is generally associated with tumors that tend to progress to late stages of the disease.[24]

In colorectal cancer, the expression of leptin is linked to a poor prognosis, and in some studies, it is also linked to the metastatic status of the tumor; hence, leptin could be a useful prognostic factor.[28] However, In our study, however, after multivariable adjustment, leptin was not an independent predictor of metastatic disease (adjusted OR=1.002; 95% CI: 0.999–1.004; p=0.128). (Table 3). Also, leptin (AUC=0.582, p=0.117) was not found to be significant after Bonferroni correction (Table 4) (Fig. 1). The discrepancies between our results and those published in previous reports might be due to differences in the number of patients, patient characteristics, clinical and pathological features, and methodological differences between the study populations. Especially, there are limited regional studies of leptin and the possibility that population specific or regional differences could account for the variation in the level of leptin and the relationships with metastatic disease.

CEA is a non-specific serum biomarker upregulated in CRC. The concentration of CEA is modulated by tumor stage, grade and other factors.[29] CEA is important to regulate promoter activity involved in cell adhesion and clustering in tissues. Therefore, it is believed to have a major role in the invasion of tumors and the metastasis of cancer to other organs.[30,31] Differences in CEA levels between metastatic and non-metastatic colorectal cancer have been consistently reported in scientific papers, with metastatic disease typically showing higher serum CEA concentrations than non-metastatic.[32] This finding is consistent with the present study.

The present study showed a statistically significant difference between both groups studied with CEA level, 90.17 pg/mL for non-metastatic group and 176.39 pg/mL for metastatic group (p<0.001) (Table 2). Moreover, Multivariable logistic regression analysis showed that metastatic disease was significantly related to CEA (OR 1.018, 95% CI 1.011–1.025, p<0.001) (Table 3). Furthermore, the highest AUC (0.802, 95% CI: 0.717–0.888) on ROC showed with CEA levels (Table 4) (Fig. 1).

These findings are supported by the literature, as many studies have demonstrated that only patients with metastatic CRC had a significant association with an elevated CEA. Studies also looking at CEA combined with other factors, and the relation to TNM stages, yielded similar results.[30] This is consistent with recent

research, which shows that Elevated CEA is seen in only 30-50% of non-metastatic CRC, but is >70-90% in metastatic CRC, related to tumor burden and liver involvement.[33]

Increased levels of CEA can be attributed to its binding to dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) that is involved in the adhesion and spreading of tumors.[34] Meanwhile, CEA inhibits the differentiation of cells through autocrine signals that promote the survival of tumor cells. It also stimulates the proliferation of endothelial cells and tumor blood vessels via paracrine mechanisms.[30] Furthermore, increased concentrations of CEA in metastatic disease and increased CEA with advancing TNM stage are consistent with increased total tumor mass and metastatic deposits being able to produce and release more CEA into the circulation. Thus, existing clinical and translational data suggest that circulating CEA is a marker of tumor burden and the CEA itself may have a role in tumor biology, including tumor survival and angiogenesis. As previously stated, CEA is a good indicator of overall survival as per guidelines.[30] In conclusion, CEA can be used to evaluate the prognosis of colorectal, especially in advanced stages of the disease, and may offer a new perspective for clinical decision making.[35] Volumetric and radiological classification of disease was not systematically recorded, but the changes in serum leptin and hepatic markers noted in our data set are probably related to the cumulative volume of disease and its tissue stress. Further prospective studies are suggested to clarify the exact link between metastatic burden and adipokine kinetics

Total protein (TP) is used to measure nutritional status, inflammation and liver function in common clinical assays.[36,37] In present study, serum total protein level in metastatic CRC patients (56.11 g/L) was found to be less than non-metastatic CRC patients (64.35 g/L) but the difference was not significant P=0.012 (Table 2). This is similar to the findings of previous study, however, to our knowledge, there has been very little research prior to this on the relationship between TP and CRC and warrants further investigation. Moreover, multivariable logistic regression analysis showed that TP was not independently associated with metastatic disease after multivariable adjustment (adjusted OR=0.980, 95% CI: 0.958–1.002, P=0.080) (Table 3). Also, the total protein level with ROC did not achieve the Bonferroni adjusted significance level (AUC=0.618, p=0.022) (Table 4) (Fig. 1).

In the present study, a decrease in the levels of total protein was observed in the metastatic group, might be align with cancer cachexia mechanisms documented in advanced malignancies. The decrease of total protein is mostly caused by systemic metabolic derangements of metastatic colorectal cancer. This is due to cachexia, liver dysfunction and treatment-induced changes in proteins, not specific to the tumor.[38,39] Therefore, our results, therefore, add further evidence that hypoproteinemia is a sign of a deep-seated systemic impairment of the patient's immune nutritional status related to the metastatic process. Even though there was a significant difference in total protein between the initial groups, this was not seen in our study after the multivariable adjustment and ROC analysis, which indicated that the apparent association with metastatic disease may be attributable to other factors and may have a limited role as a biomarker.

Aminotransferases specifically AST and ALT are common blood-based markers of liver injury.[15,16] AST, ALT, ALP and total bilirubin levels in the blood were significantly elevated in patients with metastatic CRC compared to those without metastases.

Our study demonstrated that the metastatic group exhibited significantly higher mean levels of liver enzymes compared to the non-metastatic group, including: AST: (38.83±10.21 U/L) vs. (29.70±7.01 U/L), ALT: (45.63±21.43 U/L) vs. (27.16±7.58 U/L), ALP: (131.99±40.95 U/L) vs. (96.64±30.05 U/L), $p<0.001$. Also, statistical analysis revealed that total bilirubin was significantly higher ($p<0.001$) in the metastatic group (0.862±0.778 mg/dL) compared to non-metastatic group (0.431±0.140 mg/dL) ($p<0.001$) (Table 2). Furthermore, Multivariable logistic regression analysis showed that the AST ($p<0.001$), ALT ($p<0.001$), ALP ($p<0.001$) and total bilirubin ($p=0.001$) continued to be independently associated with metastatic disease. Additionally, ROC was shown to be statistically significantly discriminatory ($p<0.001$) (Table 4) (Fig. 1), as was ALT, ALP and AST.

The results are due to the growth pattern of metastatic tumor cells in the liver: the spread of tumor cells is accompanied by compression and destruction of adjacent liver cells, which leads to decreased blood flow and to destruction of liver cells. There is a positive correlation between the number of liver enzymes (AST, ALT) in the blood and this injury, which explains the higher levels observed in the metastatic group.[40] Elevated ALP and bilirubin levels are good markers of cholestasis (blockage of bile flow from the liver). When the liver is involved with metastatic tumors, they can compress the bile ducts and lead to this obstruction. In addition, as metastatic tumors grow, they change the structure of the liver and block small bile channels. The blockages lead to an accumulation of conjugated bilirubin, which stimulates an increase in the production of ALP in cells lining the bile ducts. This is why that high level is observed in the metastatic group.[41] This finding is congruent with the results of the present study and confirms the association found between these two variables.

A recent study demonstrated that the degree of elevation in these enzymes, particularly bilirubin and ALP, may be diagnostic and a significant hyperbilirubinemia is frequently a sign of a more advanced disease.[42] Another recent studies demonstrate that these markers are helpful in assessing liver involvement and staging.[43] Our findings support this evidence. Our findings support this evidence. These enzymes are easy to measure and affordable, so they can raise suspicion of liver involvement during routine follow-up of colorectal cancer patients.

Conclusion

In conclusion, our results indicate that there are significant differences in the serum levels of CEA, AST, ALT, ALP, and bilirubin between the colorectal cancer patients. In addition, multivariable analyses and ROC assessments indicate that among the investigated parameters, CEA and liver enzymes showed the most consistent independent associations with metastatic status and the strongest discriminatory

performance. In contrast, there was no independent association of leptin or total protein with metastatic status after adjustment, and while bilirubin was associated in multivariable models, its association did not show strong discriminatory ability on ROC analysis. This observation study identifies some important systemic and metabolic correlates of metastatic disease that would require further investigation. With this one-center study, however, these markers can only be considered as prospective auxiliary markers, not as a diagnostic tool. Further, due to sample size limitations in the individual non-metastatic cohorts, patients with stage II and III colorectal cancer were not analyzed separately but as a non-metastatic group. Further large-scale, multi-center prospective studies are warranted to validate these findings and to clarify the exact independent value of these biomarkers in larger clinical cohorts.

Declarations

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Author contributions

Conceptualization, M.J.I., T.J.T., and A.H.A.; Methodology, T.J.T., W.T.S., and S.M.J.; Software, T.J.T. and W.T.S.; Validation, M.J.I., T.J.T., and A.H.A.; Formal Analysis, T.J.T. and S.M.J.; Investigation, T.J.T., W.T.S., and S.M.J.; Resources, M.J.I. and A.H.A.; Data Curation, T.J.T. and W.T.S.; Writing – Original Draft Preparation, T.J.T.; Writing – Review & Editing, M.J.I., T.J.T., A.H.A., W.T.S., and S.M.J.; Visualization, T.J.T. and S.M.J.; Supervision, M.J.I. and A.H.A.; Project Administration, M.J.I. and T.J.T.; Funding Acquisition, M.J.I. and A.H.A.

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

The data used in this study is available from the corresponding author upon reasonable request.

Ethics approval

The present study was reviewed and approved by the Research Ethics Committee of Mustansiriyah University (approval no. BCSMU/1024/0050Z).

Use of AI and AI-assisted technologies in the writing process

The authors declare that no AI or AI-assisted technologies were used in the writing or preparation of this manuscript.

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