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Potential Role of Circulating Dermokine and Bcl-2 Anti-apoptotic Protein in Colorectal Cancer Egyptian Patients: Correlative Analysis with the Clinicopathological Parameters

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Running title: *Circulating Dermokine and Bcl-2 in CRC Egyptian Patients.*

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Abstract:

Colorectal cancer (CRC) is the seventh most common cancer in Egypt, and more than half of the patients are under the age of 50. Here, we aimed to assess the levels of circulating Dermokine (DMKN) and cytoplasmic anti-apoptotic protein, Bcl-2 for detecting CRC in the earlier stages possible. The levels of DMKN, Bcl-2, Carcinoembryonic antigen (CEA), and Carbohydrate antigen 19.9 were determined using ELISA in the sera of 53 CRC patients, 18 ulcerative colitis patients, and 24 healthy individuals. Statistical analyses were performed using the SPSS program. Serum levels of DMKN and Bcl-2 were significantly higher ($p < 0.0001$) in CRC patients than in non-cancer individuals. Highly significant correlations were recorded between levels of DMKN and Bcl-2 and the pathological TNM tumor characteristics. At the best cut-off level (68-pg/mL), the DMKN assay showed high degrees of sensitivity (87%), specificity (100%), and accuracy (91%) in comparison with investigated biomarkers. Furthermore, regression analysis revealed a DKB-Score based on DMKN and Bcl-2 with an AUROC of 0.991. The developed score showed a high degree of efficiency (97.4%) for discriminating CRC patients from controls. In conclusion, the assessment of serum DMKN either alone or simultaneously with Bcl-2 has a potential role in discriminating CRC from premalignant patients.

Keywords: *Dermokine, Bcl-2, Ulcerative colitis, Colorectal cancer, Egypt.*

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1. Introduction

Colorectal cancer (CRC) is the second biggest cause of cancer-related deaths, which is a common malignant cancer that affects people of all ages and has a poor prognosis as the disease progresses **(Bopanna et al., 2017)**. It is known that long-standing ulcerative colitis (UC) leads to CRC and is often a threat to patients' lives **(Molodecky et al., 2012)**. Rapid development, invasiveness, and significant treatment resistance are characteristics of CRC **(Jelski and Mroczko, 2020)**. Although early-stage CRC patients typically have a positive outlook, those with metastatic disease to lymph nodes (stage III) or distant organs (stage IV) have a significantly higher recurrence and mortality rates. Therefore, about 50% of patients have advanced disease when they first arrive, necessitating multimodal therapy, which includes surgery and chemotherapy **(Howlader et al., 2021).** Diagnosing CRC at an early stage is not easy as cancer is often asymptomatic **(Jelski and Mroczko, 2020)**. Unfortunately, the most accurate invasive colonoscopy and the most popular noninvasive fecal occult blood test (FOBT) both have drawbacks and poor sensitivity levels **(Sung et al., 2008; Brenner et al., 2011)**. Carcinoembryonic antigen (CEA), carbohydrate antigen 19.9 (CA 19.9), and tissue polypeptide specific antigen (TPS) have all been utilized as traditional indicators to identify CRC **(You et al., 2019)**. However, none of these tests can improve the early detection of colorectal cancer or have excellent diagnostic accuracy **(Stiksma et al., 2014; Jelski and Mroczko, 2020)**. Cancer-related cell products, such as circulating tumor cells (CTCs), cell-free circulating nucleic acids (Cf DNA/RNA), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), exosomes, and proteins from the primary or metastatic tumor, are released into the extracellular environment by cancer cells because of their high rates of turnover. These biomarkers are recognized to provide significant information on physiological processes at the single-cell level and are released either directly or indirectly into body fluids like cerebrospinal fluid, ascitic fluid, pleural effusion, peripheral blood, urine, and saliva **(Fernandez-Lazaro et al., 2020)**. Key apoptotic regulators in the B-cell lymphoma-2 (Bcl-2) family of proteins, whose dysregulation can have a variety of pathogenic effects, including the emergence of cancer **(Czabotar et al., 2014)**. The Bcl-2 protein is a 26-kDa protein that blocks apoptosis and inhibits programmed cell death **(Bhardwaj et al., 2020)**. In reality, evading apoptosis is frequently linked to dysregulation of the anti-apoptotic protein Bcl-2 **(Delbridge et al., 2016)**. However, evasion of apoptosis is associated with treatment resistance and metastasis of CRC **(Faruk et al., 2021)**. A new stratified epithelium-secreted gene complex (SCC) formed by dermokine (DMKN) (sk30/89) and two additional keratinocyte-secreted peptides was also discovered **(Naso et al., 2007; Toulza et al., 2006)**. The secreted DMKN is highly expressed in differentiated layers of stratified epithelia of the colon **(Naso et al., 2007; Toulza et al., 2006; Moffatt et al., 2004)**. The human-expressed sequencing tag database has classified DMKN as a cancer-expressing gene in addition to its expression in normal multilayered epithelia **(Toulza et al., 2006).** Recently, we investigated the potential association of CD133 and CD44 cell surface biomarkers as independent predictors in CRC development among Egyptian patients **(El-Emshaty et al., 2019, 2021).** Here, we investigated the diagnostic performance of circulating DMKN, and Bcl-2 protein expression compared with the classical biomarkers (CEA and CA19.9) and considers their correlative analysis with the clinicopathological variables of CRC patients.

2. Subjects and Methods.

2.1. Study population.

This prospective study included 95 eligible Egyptian individuals; 53 patients with CRC (25 males and 28 females, mean age 57.7 ranged from 31-83 yr) and 42 non-cancer individuals [18 with UC (11 males and 7 females, mean age 50.3 ranged from 24-67 yr) and 24 healthy individuals (10 males and 14 females, mean age 51.1 ranged from 33-81 yr)]. The CRC

patients were diagnosed based on standard clinical endoscopic, histologic, and radiographic criteria. International Union Against Cancer (UICC) and the TNM Classification of TNM staging criteria were followed **(Gospodarowicz et al., 2008).** The tumor was identified in 51% of CRC patients in ascending (right) colon, in 26% in descending (left) colon, in 4% at the sigmoid, and in19% at the rectum. The exclusion criteria were patients with other cancers, gastrointestinal tract complications other than colon, high blood lipid, familial adenomatous polyposis, or previous history of malignancy. Healthy individuals were selected without symptoms or signs of other colorectal diseases and a clinical history of hepatitis or malignancy. Blood samples of all patients and healthy controls were collected at Gastrointestinal Surgery Center (GISC), Mansoura University, Egypt during the period from March 2021 to February 2022, and sera of all cases were stored at - 70 ºC until used. All participants gave written informed consent, the study was conducted in compliance with the Declaration of Helsinki, and it was approved by Mansoura University's GISC Ethics Committee. The clinicopathological data of CRC patients are listed in **Table 1.**

2.2. Measurement of serum CEA, CA19-9, Bcl-2, and DMKN

Serum levels of biomarkers were measured by enzyme-linked immunosorbent assay (ELISA). Serum samples were tested in duplicates using sandwich ELISA for CEA (ELISA Kit, Catalogue No. E-OSEL-H0016, Elabscience, Houston, Texas, USA), CA19-9 (EIA kit, Catalogue No. TM E-4500, LDN, Labor Diagnostika, Nord GmbH Co. KG, Germany), Bcl-2 and DMKN ELISA kits (Glory Science Co., Ltd, 2400 Veterans Blvd. suite 16-101, Del Rio, TX 78840, USA) according to the instructions of the manufacturers.

2.3. Statistical Analysis

The Kolmogorov-Smirnov test was used to determine whether continuous data were normal. When continuous data are not normally distributed, they are given as medians (25-75th percentiles), or as the mean and standard deviation for normally distributed data. Data with categories are expressed as counts (percentages). Unpaired Student's t-test and Mann–Whitney U test were used to compare the data between the two groups. Categorical variables were compared using the Chi-square test between different groups. Spearman correlation analysis was used to determine the correlation between investigated non-parametric data. The capacity of the examined blood biomarkers to forecast CRC disease was assessed using receiver operating characteristic (ROC) curve analysis. To study the relationship between the blood levels of the several examined biomarkers, simple linear regression analysis was utilized. All the data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, Illinois), and a two-sided p-value < 0.05 was considered statistically significant.

3. Results

3.1. Serum levels of DMKN, Bcl-2, CEA, and CA19-9 in all study individuals

Serum levels of CEA, CA19-9, Bcl-2, and DMKN in CRC patients and non-malignant individuals (UC and healthy individuals) were listed in **Table 2**. The median DMKN serum level in CRC patients (88.14 pg/mL) was significantly higher than that in UC patients (64.41 pg/mL) and healthy controls (42.38 pg/mL), $P < 0.0001$. Serum levels of Bcl-2, CEA, and CA19-9 were also significantly elevated in CRC patients compared with UC and healthy individuals (Bcl-2: 40 vs 30.02 and 20.51 ng/mL, $P < 0.0001$; CEA: 7.0 vs 9.0 and 2.0 ng/mL, $P < 0.0001$; CA19-9: 16.5 vs 5 and 3 U/mL, $p < 0.0001$). CEA and DMKN levels were also elevated significantly in UC compared to healthy individuals ($P < 0.0001$), however, no significant difference was recorded between UC and healthy individuals using CA19-9 ($P > 0.05$).

3.2. Correlation between serum CEA, CA19-9, Bcl-2, and DMKN with the Clinicopathological variables of CRC.

The relations between serum CEA, CA19-9, Bcl-2, and DMKN levels and the clinicopathological variables of CRC patients were listed in **Table 1**. CA19-9 overexpression showed a significant correlation with the pathological-M-Stage (r = 0.251, *P* = 0.035), pathological-N-stage (r = 0.227, *P* = 0.051), advanced stage (III and IV) of CRC (r = 0.229, *P* = 0.05). The CRC patients with a lower stage (I and II) had substantially lower CA19-9 concentrations compared to those with advanced stages (III and IV) of CRC (r = 0.229, *P* = 0.05). CEA had substantially lower levels in CRC patients with a lower stage (I and II) compared to those with advanced stages (III and IV) of CRC (r = 0.269, *P* = 0.026). As regards Bcl-2, a significant correlation was recorded with age (r = 0.253, *P* = 0.034), pathological-T-stage (r = 0.483, *P* < 0.0001), pathological-N-stage (r = 0.499, P < 0.0001), pathological-M-Stage (r = 0.381, p = 0.002), clinical stage (r = 0.449, *P* < 0.0001) and the CRC patients with a lower stage (I and II) had substantially lower Bcl-2 concentrations compared to those with advanced stage (III and IV) (r = 0.412, *P* = 0.001), and the CRC patients with lower grade (I) had substantially lower Bcl-2 concentrations compared to those with advanced grade (II and III) of CRC (r = 0.298, *P* = 0.015).

DMKN was significantly correlated with tumor size (r = 0.280, *P* = 0.021), pathological Tumor Size (pT) (r = 0.31, *P* = 0.013), histologic grade (r = 0.49, *P* < 0.0001), The CRC patients with lower grade (I) had substantially lower DMKN concentrations compared to those with advanced grade (II and III) of CRC (r = 0.357, *P* = 0.004), pathological-T-stage (r = 0.463, *P* < 0.0001), pathological-N-stage (r = 0.338, *P* = 0.007), pathological-M-Stage (r = 0.318, *P* = 0.01), clinical stage (r = 0.325, *P* = 0.009); CRC patients with a lower stage (I and II) had substantially lower DMKN concentrations compared to those with advanced stage (III and IV) of CRC (r = 0.292, *P* = 0.017).

3.3. The Diagnostic Utility of Examined Biomarkers Using the ROC Curve

Figure 1 depicts the possible diagnostic role of the DMKN, Bcl-2, CEA, and CA19-9 markers in CRC as determined by the ROC curve and AUC. According to the ROC analysis, the optimal cut-off values for DMKN, Bcl-2, CEA, and CA19-9 were 68 pg/mL, 26 ng/mL, 3 ng/L, and 37 U/L; respectively. The ROC curve showed that DMKN had superior sensitivity, specificity, and accuracy for differentiating CRC patients from healthy controls at a threshold of 68 pg/mL, with an AUC of 0.972 **(Figure 1).** Stepwise linear regression analysis of investigated biomarkers developed a DKB-Score based on DMKN and Bcl-2 markers as follows:

DKB-Score = 0.195 + (DMKN *0.003) + (Bcl-2 *0.006)

The correlations between the DKB-score and the clinicopathological variables of CRC patients were listed in **Table 3**. DKB-Score analysis showed a significant correlation with the pathological-T-stage (r = 0.565, *P* < 0.0001), pathological-N-stage (r = 0.525, *P* < 0.0001), pathological-M-Stage (r = 0.412, *P* = 0.001), histologic grade (r = 0.336, *P* = 0.007), The CRC patients with lower grade (I) had substantially lower (DKB Score) concentrations compared to those with advanced grade (II and III) of CRC (r = 0.327, p = 0.008) and clinical stage (r = 0.486, p < 0.0001). The CRC patients with a lower stage (I and II) had substantially lower DKB-Score concentrations compared to those with advanced stages (III and IV) of CRC (r = 0.436, p = 0.001). The DKB-Score with a cut-off of 0.511 showed a sensitivity of 96.2%, specificity of 100%, and accuracy of 97.4% for discriminating CRC patients from healthy controls with AUC 0.991 **(Table 4).**

Table 1. Relation between clinicopathological features of 53 patients with colorectal cancer and serum levels expressed as median (25 – 75th percentiles) of investigated biomarkers.

Clinicopathological feature	N	%	CEA[≠] (ng/mL)	CA19.9 (U/mL)	Bcl-2* (ng/mL)	DMKN (pg/mL)
Sex						
Male	**25**	47.2	4.2 (1.9 - 40.7)	16.5 (4.9 - 86.0)	34.7 (30.2- 70.0)	89.5 (71.9 - 106.4)
Female	**28**	52.8	7 (3.2 - 40.0)	15.85 (5.1 - 55.2)	40 (30.3 - 80.0)	86.8 (75.2 - 115.3)
Age (yr)*						
≤ 50	**17**	32.1	8 (3.2 - 40.4)	21 (4.3 - 151.5)	32.0 (25.6 - 50.0)	91.5 (80.4 - 111.9)
> 50	**36**	67.9	6.9 (2.6 - 40.7)	15.9 (5.1 - 55.9)	60 (31.6 - 80.0)	86.9 (71.4 - 107.0)
Tumor Size (mm)						
≤ 50	**32**	60.4	7.5 (3.0 - 40.5)	18.7 (4.6 - 68.3)	50 (28.8 - 60.0)	84.8 (71.4 - 102.4)
> 50	**21**	39.6	5 (2.4 - 52.5)	14.8 (5.5 - 83.5)	34.7 (31.7 - 80.0)	102.4 (81.4 - 134.3)
Histologic grade [#],*						
GI	**6**	11.3	5.5 (1.9 - 45.5)	12.6 (5.9 - 16.5)	21.8 (21. 5 - 60.0)	72.9 (54.7 - 80.9)
GII	**31**	58.5	6.1 (2.7 - 38.0)	17.3 (4.5 - 63.0)	40 (32.0 - 80.0)	85.6 (72.0 - 105.1)
GIII	**16**	30.2	10.3 (3.1 - 71.1)	19.3 (5.2 - 186.3)	50 (31.7 - 80.0)	102.4 (90.3 - 193.8)
Low-grade (GI)	**6**	11.3	5.5 (1.9 - 45.5)	12.6 (5.9 - 16.5)	21.9 (21.5 - 60.0)	72.9 (54.7 - 80.9)
High grades (GII, GIII)	**47**	88.7	7 (2.8 - 40.7)	17.3 (5.0 - 85.0)	40 (31.7 - 80.0)	97.5 (74.0 - 118.6)
Pathological T-Stage *						
T1	**5**	9.4	4 (2.0 - 50.4)	15.3 (10.3 - 507.5)	25.8 (21.4 - 33.4)	74.2 (70.3 - 91.1)
T2	**11**	20.8	4 (2.0 - 38.0)	20 (4.5 - 34.9)	32.0 (29.0 - 40.0)	84.8 (55.9 - 102.4)
T3	**26**	49.1	7.5 (3.4 - 40.7)	17.1 (4.9 - 73.0)	60 (31.3 - 80.0)	85.2 (72.6 - 105.1)
T4	**11**	20.8	11 (2.7 - 72.0)	5.6 (2.8 - 115.0)	80 (40.0 - 100.0)	150 (102.4 - 175.0)
Pathological N-Stage*						
Negative lymph node	**21**	39.6	4.2 (1.8 - 39.0)	10.7 (2.8 - 28.2)	31.7 (21.9 - 50.0)	77.9 (68.6 - 102.4)
Positive lymph node	**32**	60.4	7.8 (3.1 - 44.0)	18.2 (5.98 - 86.5)	60 (32.0 - 80.0)	101.2 (81.5 - 142.2)
Pathological M-Stage [#]						
No Metastasis	**34**	64.2	5.2 (2.5 - 38.5)	12.75 (4.4 - 34.2)	32.0 (23.7 - 60.0)	82.0 (71.2 - 105.1)
Metastasis	**19**	35.8	12.2 (2.8 - 62.0)	62 (8.0 - 115.0)	60 (32.0 - 80.0)	102.4 (85.6 - 150.0)
Clinical stage *						
Stage I	**12**	22.6	5.6 (1.8 - 40.5)	18.65 (7.9 - 85.3)	31.8 (22.7 - 38.7)	81.4 (69.9 - 101.2)
Stage II	**11**	20.8	3.7 (1.5 - 11.0)	4.7 (2.0 - 15.3)	32.0 (22.0 - 60.0)	79.6 (67.8 - 150.0)
Stage III	**12**	22.6	8.8 (4.0 - 43.2)	20.1 (5.1 - 79.5)	60 (32.0 - 95.0)	93.9 (72.2 - 142.2)
Stage IV	**18**	34	10.1 (2.78 - 61.3)	39.7 (7.4 - 138.8)	60 (31.9 - 80.0)	101.2 (85.4 - 127.7)
Low stages (I + II)	**23**	43.4	4.2 (1.5 - 38.0)	10.7 (2.9 - 22.3)	32.02 (22.0 - 60.0)	79.7 (69.5 - 102.4)
High stages (III + IV)	**30**	56.6	9 (3.9 - 47.5)	20.1 (5.5 - 92.5)	60 (31.9 - 80.0)	101.2 (80.3 - 126.5)

Data are presented as Median (25-75th percentiles).

**** Indicates a significant Spearman correlation between data of CA19-9, CEA, Bcl-2, and DMKN.***

 # Indicates a significant difference (p < 0.05) between data of CA19-9, CEA, Bcl-2, and DMKN.

Table 2. Serum levels of CEA, CA19-9, Bcl-2, and DMKN biomarkers in the different study groups

Biomarker*	CRC patients (n = 53)	UC patients (n = 18)	Healthy individuals (n = 24)	P value**
CEA (ng/mL)	7 (2.75 - 40.7)	9 (5.5 - 28.5)	2 (1.4 - 3.0)	< 0.0001
CA 19.9 (U/mL)	16.5 (5.0 - 76.0)	5 (3.0 - 11.4)	3 (2.9 - 6.0)	< 0.0001
Bcl-2 (ng/mL)	40 (30.9 - 80.0)	30.0 (25.8 - 34.7)	20.5 (19.0 - 23.4)	< 0.0001
DMKN (pg/mL)	88.1 (74.5 - 106.4)	64.4 (57.9 - 79.7)	42.4 (25.0 - 55.7)	< 0.0001

Data are presented as Median (25-75th percentiles).

***** P value < 0.05 is considered significant and the difference between groups was calculated using Kruskal -Wallis Test.***

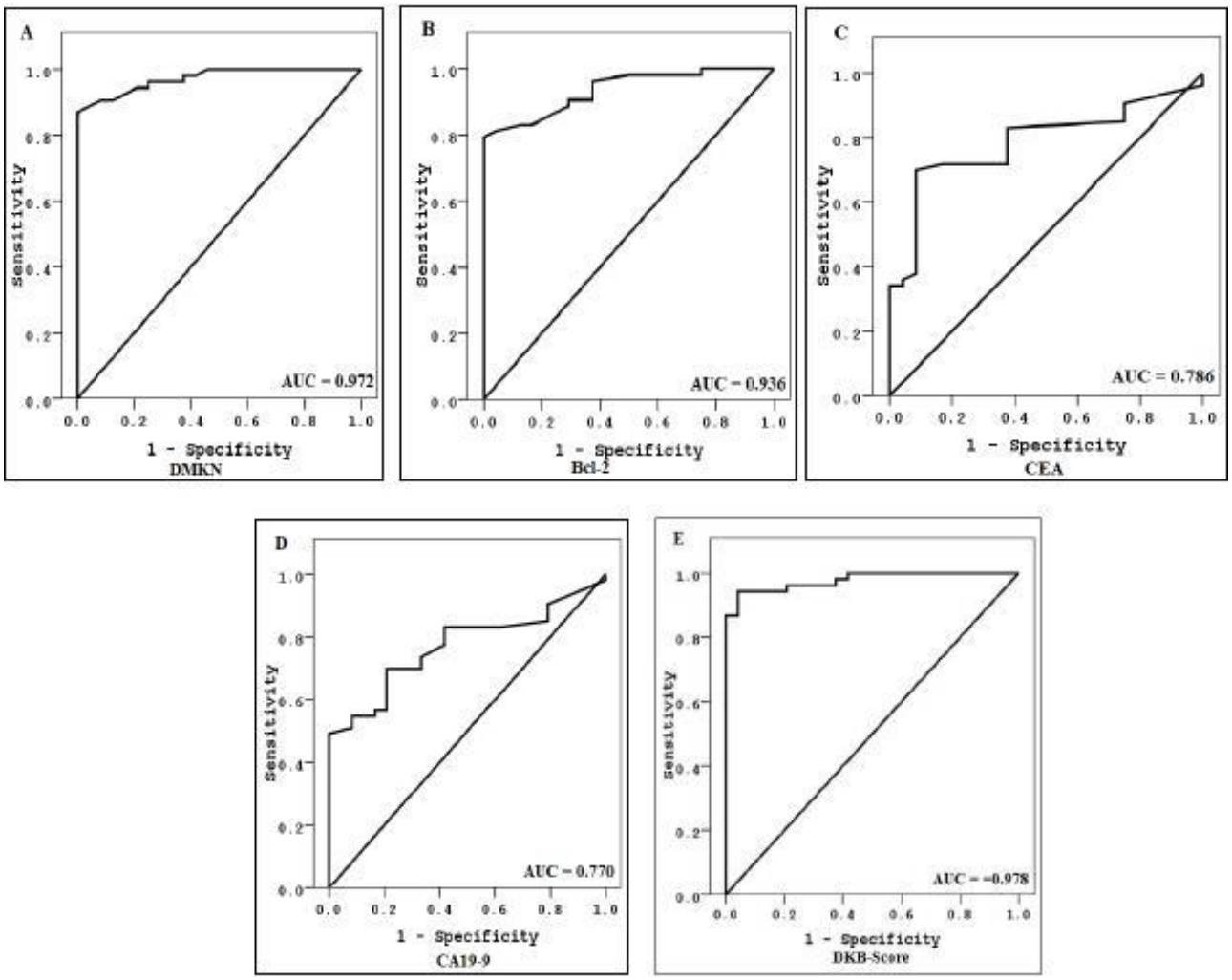

Figure 1: Receiver operating characteristic (ROC) curve analysis using four investigated biomarkers to differentiate CRC patients. The diagnostic accuracy of each biomarker was determined by obtaining the largest possible area under the ROC curve (AUC). A. DMKN, B. Bcl-2, C. CEA, D. CA19-9, and E. DKB-Score.

Table 3. Relationship between the clinicopathological features of colorectal cancer patients and the developed DKM-score. Data are presented as Median (25-75th percentiles).

Clinicopathological feature	N	%	DKB-Score
Sex			
Male	**25**	47.2	0.67 (0.58 - 0.86)
Female	**28**	52.8	0.69 (0.61 - 0.93)
Age (yr)*			
≤ 50	**36**	32.1	0.66 (0.58 - 0.78)
> 50	**17**	67.9	0.71 (0.61 - 0.93)
Tumor Size (mm)			
≤ 50	**32**	60.4	0.67 (0.58 - 0.85)
> 50	**21**	39.6	0.67 (0.62 - 1.0)
Histologic grade #,*			
GI	**6**	11.3	0.55 (0.49 - 0.68)
GII	**31**	58.5	0.67 (0.59 - 0.86)
GIII	**16**	30.2	0.76 (0.64 - 1.2)
Low grade (GI)	**6**	11.3	0.55 (0.49 - 0.68)
High grades (GII, GIII)	**47**	88.7	0.71 (0.64 - 0.93)
Pathological T-Stage *			
T1	**5**	9.4	0.58 (0.55 - 0.59)
T2	**11**	20.8	0.65 (0.51 - 0.67)
T3	**26**	49.1	0.71 (0.62 - 0.90)
T4	**11**	20.8	1.0 (0.75 - 1.245)
Pathological N-Stage*			
Negative lymph node	**21**	39.6	0.58 (0.53 - 0.67)
Positive lymph node	**32**	60.4	0.76 (0.66 - 0.99)
Pathological M-Stage #			
No Metastasis	**34**	64.2	0.66 (0.55 - 0.81)
Metastasis	**19**	35.8	0.77 (0.66 - 1.1)
Clinical stage *			
Stage I	**12**	22.6	0.59 (0.55 - 0.66)
Stage II	**11**	20.8	0.58 (0.51 - 1.0)
Stage III	**12**	22.6	0.76 (0.66 - 0.93)
Stage IV	**18**	34	0.76 (0.66 - 1.0)
Low stages (I + II)	**23**	43.4	0.59 (0.54 - 0.67)
High stages (III + IV)	**30**	56.6	0.76 (0.66 - 0.95)

**** Indicate a significant Spearman correlation between data of DKB-score.***

 # Indicate a significant difference (p < 0.05) between data of DKB-score.

Table 4: The performance characteristics of serum CA19-9, CEA, Bcl-2, and DMKN biomarkers in comparison with the developed DKB-score for laboratory diagnosis of CRC Egyptian patients

Test variable	Sensitivity (%)	Specificity (%)	Efficiency (%)
CA19-9	**32**	**100**	**53**
CEA	**72**	**83**	**75**
Bcl-2	**81**	**96**	**86**
DMKN	**87**	**100**	**91**
DKB-Score	**96**	**100**	**97**

4. Discussion

Colorectal cancer ranks third in terms of newly diagnosed cases (10%) and is the second biggest cause of cancer-related deaths globally, with 9.4% **(Sung et al., 2021).** CRC is Egypt's seventh most prevalent cancer accounting for 4.2 % of men and 3.8 % of women (**Ibrahim et al., 2014**) with a male-to-female ratio was 1:1 (**Bhardwaj et al, 2020, Ghavam-Nasiri et al., 2007**) comparable to a male-to-female ratio (0.89) in the current study. The incidence rates of CRC in young people have grown since the early 1990s, from 8.6 per 100,000 in 1992 to 12.5 per 100,000 in 2015, a rise of 45% overall **(Seigel et al., 2014, Murphy et al., 2019**). CRC patients in our study aged from 31-83 years with 32.1% were in younger people (aged ≤ 50 years**).** But because CRC is a disease that can be prevented and treated if caught early, it is critical to find non-invasive methods with high specificity and sensitivity to aid in its early identification, prognosis, and treatment monitoring **(Raza et al., 2022).** Therefore, we aimed to evaluate the circulating CEA, CA19-9, Bcl-2, and DMKN protein expression with insight into the diagnostic performance and early prediction for CRC tumor development. Serum tumor markers such as carcinoembryonic antigen and carbohydrate antigen 19–9 is commonly used for treatment monitoring **(Thrumurthy et al., 2016; American Cancer Society, 2020)**. However, CA19-9 was detected as less sensitive than CEA (**Vukobrat-Bijedic et al., 2013**). Higher Dukes' stage of the disease is accompanied by a rise in CA19-9 concentration and sensitivity, although these factors are unrelated to the location of the tumor and the number of positive lymph nodes (**Filella et al., 1992**). Similarly, CA 19-9 was highly expressed in CRC patients compared to control subjects but there were no considerable changes concerning the clinicopathological variables of CRC patients except the pathological tumor size ($P = 0.038$). According to current recommendations, CEA is the most significant serum tumor marker for the prognosis and therapeutic outcome of CRC (**Provenzale et al., 2018**). Serum CEA levels were strongly demonstrated in the CRC patients in our study and were significantly correlated with tumor grade ($P = 0.036$) and pathological M stage ($P = 0.029$). Therefore, high preoperative CEA values could

indicate a bad outcome (**Jelski and Mroczko, 2020**). According to **Tan et al. (2009)**, a quantitative meta-analysis of 20 trials comprising 4285 patients and examining the effectiveness of CEA in diagnosing CRC recurrence came to the following conclusions. The overall sensitivity of 64 % and specificity of 90 % are comparable to our results displayed the overall sensitivity and specificity of CEA were 72% and 83% respectively. The effectiveness of CEA detection for prognosis prediction and monitoring CRC patients, however, is still debatable. Its limitations include very poor sensitivity and specificity, which render it insufficient for screening large numbers of asymptomatic patients alone (**El-Awady et al., 2009**). Therefore, finding new biomarkers for CRC and validating them would be of the highest clinical significance in regular healthcare for the general public and postoperative surveillance for patients who have surgery (**Wang et al., 2017**). Liquid biopsy is the term for the examination of extracellularly produced biomarkers in bodily fluids; its significance in cancer screening, patient stratification, and monitoring has been well-documented. The significance of liquid biopsy is emphasized in the context of CRC because this tumor is highly diverse and requires molecular characterization for efficient monitoring and management **(Raza et al., 2022).** The biomolecular aspects of CRC have raised great interest in Bcl-2 gene function (**Koehler et al., 2013**). Considerable expression of Bcl-2 was recorded in presently studied CRC patients compared to non-tumor individuals ($p < 0.0001$) and related to younger age $\leq$ 50 years ($P = 0.027$), tumor grading ($P = 0.047$), pathological T stage ($P < 0.0001$), pathological N stage ($P < 0.0001$), pathological M stage ($P = 0.001$) and clinical stage ($P < 0.0001$). On the contrary, **Bhardwaj et al. (2020)** reported that Bcl-2 positivity decreases with increasing tumor stage ($P = -0.04$). Bcl-2 overall sensitivity, specificity, and efficiency for CRC diagnostic potential was 81%, 96%, and 86% respectively which were comparatively higher than that for CEA. Though, the creation of drugs that can suppress the action of Bcl-2 family members that are overexpressed in a variety of cancers, including CRC, has received considerable attention **(Besbes et al., 2015; Xu et al., 2016**). **Tagi et al. (2010)** revealed that the secreted DMKN isoforms are abnormally abundant in CRC, looked into the possibility of serum DMKN as a new biomarker, and looked into the benefits of multimarker testing for the diagnosis of early CRC. They found relatively high (33.3%) serum DMKN in early CRC patients. As a result, they suggest that the transient production of DMKN was a precursor to malignancy. In the current study, DMKN serum expression levels were significantly elevated in CRC patients compared to UC and healthy controls. The levels of DMKN were likewise linked to clinical stages and tumor differentiation in the current study but not to gender. The level of DMKN was correlated with tumor stage ($r = 0.298$, $P = 0.03$) and tumor grade ($r = 0.487$, $P < 0.0001$). However, tumor staging is the best prognostic indicator of outcome in CRC. Consistent with other studies (**Singh et al., 2020**), our results confirm that high levels of preoperative DMKN biomarkers are associated with advanced tumors including T and AJCC stages. In regular medical practice, however, the examination of a single marker in the detection and prognosis of the disease is appropriate but frequently accompanied by low sensitivity and specificity. To maximize the diagnostic value of the markers, it appears that simultaneously determining at least two or more markers is the ideal approach (**Jelski and Mroczko, 2020**). The potential diagnostic role of CEA, CA19-9, Bcl-2, and DMKN biomarkers in CRC was performed by ROC curve and AUC analyses. The present study demonstrated that CEA alone failed to discriminate CRC patients from UC (AUC 0.462, $P = 0.629$) with poor accuracy (56%) but Bcl-2 distinguished CRC from UC (AUC = 0.704, $P = 0.002$) with better accuracy (66%) than CEA. Also, the ROC curve for DMKN demonstrated optimal sensitivity, specificity, and accuracy of 87%, 100%, and 91%; respectively in distinguishing CRC patients from healthy controls at a threshold of 68 pg/mL and

AUC of 0.972. The developed DKB-Score based on values of DMKN and Bcl-2 with a cut-off of the value of 0.511 showed a sensitivity of 96.2%, specificity of 100%, and accuracy of 97.4% for discriminating CRC patients from healthy controls with AUC 0.991. The above findings indicate that the DKB-Score serum assays may provide a useful tool for the early detection of CRC because no trustworthy independent biomarker has yet been discovered for CRC screening. In conclusion, serum DMKN either alone or simultaneously with circulating Bcl-2 marker (DKB-Score) could thus have a potential role as a clinical predictor in the diagnosis and outcomes of CRC patients. However, to further confirm these promising results an additional large population and multi-central studies are still required.

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Data Availability

The corresponding author can provide the original data upon request (Email: elemshaty_h@yahoo.com).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

HI, and HME designed this study. MMH performed medical and clinical examinations. HME and SMA collected clinical data. SMA and OAO performed lab investigations. HME and HI analyzed the data and wrote the manuscript. All authors revised the manuscript and provided the final approval.

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