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ORIGINAL RESEARCH

Evaluation of Fecal M2PK as a Diagnostic Marker in Colorectal Cancer

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Abstract

Background Invasive colonoscopy is the gold standard for patients at risk for colorectal cancer. However, the need for non-invasive and specific markers is required.

Objective To evaluate the sensitivity of the glycolytic pyruvate kinase isoenzyme type M2 dimer (M2PK) as a diagnostic biomarker for colorectal cancer (CRC) and adenomatous colorectal polyps (CRP) screening.

Design Case-control.

Patients Twenty patients with CRC, 20 patients with CRP (lack criteria for colonic cancer by biopsy), and 20 normal subjects.

Outcome Complete blood count (CBC), erythrocyte sedimentation rate (ESR), tumor markers: carcino embryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), fecal occult blood test (FOBT), and fecal M2PK. Pelvic and abdominal ultrasound (US), colonoscopy, and a histopathological examination.

Results Only weight loss and cachexia were significantly associated with CRC than CRP or control groups. M2PK was the most sensitive and specific test in differentiating CRC from CRP and the control subjects (sensitivity = 75%, specificity = 100%).

Limitations (1) The selection of cases for three well-matched groups, as to perform colonoscopy in well-prepared cases and conditions. (2) Replicates in more than 20 cases for confirmation at the expense of enrolling new patients. (3) The cost associated with tumor markers analysis.

Conclusion Fecal M2PK can be used as a precolonoscopy screening test for CRC patients, and is superior to other tumor markers, and in indicating the progress of colorectal adenomas > 1 cm. Thus being cost-effective and easy-to-perform test, it is a feasible tool to preselect patients who require colonoscopy.

Keywords Fecal occult blood test · Patients · Colorectal cancer · Colorectal polyp · Marker · Egypt

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Background

In Egypt, according to National Cancer Institute statistics, CRC ranks the sixth most common cancer in males [1, 2]. Although colonoscopy is the gold standard for the early detection of CRC and its precancerous lesions, it has its risks and limitations and is associated with several complications [3–6]. Thus, non-invasive screening is of utmost importance as it is widely available, cost-effective, and has a good patient compliance [7, 8].

In contrast to colonoscopy, the FOBT [9] is widely accepted non-invasive test. FOBTs are based on the premise that polyps and cancers bleed more than normal mucosa [7]. Consequently, nonbleeding colorectal tumors or polyps and those not consistently discharging sufficient blood into the intestinal lumen are not detected by either guaiac-based or immunological FOBTs. Fecal immunochemical testing (FIT or iFOBT) is a newer, more sensitive screening method than the traditional FOBT. It utilizes specific antibodies to the globin component of the hemoglobin [10]. The sensitivities of these tests are limited [8].

Adenomas or colorectal cancer are usually associated with increased serum and stool levels of tumor M2PK [11, 8]. Fecal M2PK detects both bleeding and nonbleeding tumors and adenoma. Fecal M2PK does not have false positive results due to various noncancerous sources of bleeding, e.g., hemorrhoids and fissures. In contrast to the FOBT, only one small stool sample (from a single stool passage) is needed, and without dietary restrictions [12].

We aim at evaluating the sensitivity of M2PK as a diagnostic biomarker for CRC and adenomatous CRP screening.

Patients and Methods

The objective of the case-control study and the possible complications were explained to all patients who met the eligibility criteria. Informed consent was obtained from each participant before recruitment in the study. Eligible patients were recruited from the outpatient clinics and endoscopy units where the objectives of the study and the possible complications were explained. As well, an informed written consent was obtained from all subjects.

The study was conducted in June 2016, and the follow-up period for 6 months. The study recruited 60 Egyptian patients. Patients were divided into three groups: CRC group included 20 patients with CRC confirmed by colonoscopy and biopsy. CRP group included 20 patients with colonic polyps that lack criteria for definite colonic cancer by colonoscopy and biopsy. The control group included 20 patients with normal or nonspecific colonoscopy findings (other than polyps or definite cancers). The studied groups were age- and sex-matched.

Exclusion criteria Patients with previous surgical resection of a colonic mass, and patients on chemotherapy were excluded. Also, patients in whom colonoscopy was not completed due to any cause (bad preparation, intolerability, etc.) and patients with inflammatory bowel disease either ulcerative colitis or Crohn's disease were excluded.

Patients and control groups were subjected to the following: (i) history taking and clinical evaluation. FOBT in stool, pelvic-abdominal US, and digital rectal exam were done for all subjects. A colonoscopy, colon biopsy, and histopathology to assess CRC, defined by an invasion of malignant cells beyond the mucosa on the pathology report (unpublished data), were also done. Histological classification of total polyps including adenomatous (tubular, tubulovillous, or villous and the degree of dysplasia whether high or low-grade dysplasia) and non-adenomatous polyps was done [13].

Data Availability The corresponding author can be contacted if someone wishes to request the raw de-identifiable data. All data generated or analyzed during this study are included in this published article.

Laboratory Investigations

Hematological Indices

Fresh blood samples were used for ESR determination using the Wintrobe method [14]. CBC was determined by Cell Dyne 1800. Blood hemoglobin (Hb) concentration was determined by photometric quantification of cyanmethemoglobin at 540 nm [15].

Prothrombin Time (PT) and Concentration

Fresh plasma is used for PT measurement and concentration using blood coagulometer model SEAC S2, Biostec Liquiplstin, Egypt. Normal value (12–14 s), up to 75% in concentration, and an international normalization ratio (INR, the ratio of the patient's PT and the normal mean PT) is up to 1 [16].

Tumor Markers (CEA and CA19-9)

Tumor markers CA19-9 and CEA were determined using fresh blood. An immunoradiometric method with a commercially available CA19-9 radioimmunoassay (RIA) diagnostic kit (Enzyme Linked Immunosorbent Assay (ELISA-CA19-9, CIS Bio International, France)) and CEA RIA diagnostic kit (CIS Bio Industries, Gif-Sur-Yvette, France) was performed. Cutoff values recommended for diagnostic purposes were 37 kU/L for CA19-9 and 22 μg/L for CEA. Values above the cutoff concentrations were considered positive.

Table 1 Age, gender, medical history and clinical presentations among the studied groups

Variables		CRC ($N = 20$)	CRP ($N = 20$)	Ctrl ($N = 20$)	CRC/CRP	CRC/Ctrl	CRP/Ctrl
Age (Years)	Range	29.0 – 67.0	26.0 – 67.0	31.0 – 69.0	0.774^	0.742^	0.967^
	Mean ± SD	52.6 ± 9.4	51.6 ± 11.2	51.5 ± 11.5			
Gender (*n* %)	Female	6 (30.0%)	4 (20.0%)	7 (35.0%)	0.465#	0.736#	0.288#
	Male	14 (70.0%)	16 (80.0%)	13 (65.0%)			
Medical History	Family history	2 (10.0%)	0 (0.0%)	0 (0.0%)	0.147#	0.147#	1.000#
	Smoking	11 (55.0%)	10 (50.0%)	7 (35.0%)	0.752#	0.204#	0.337#
	DM	10 (50.0%)	7 (35.0%)	7 (35.0%)	0.337#	0.337#	1.000#
	HTN	4 (20.0%)	5 (25.0%)	4 (20.0%)	0.705#	1.000#	0.705#
Clinical Presentations	Symptoms						
	Weight loss	13 (65.0%)	1 (5.0%)	3 (15.0%)	*< 0.001**#	*< 0.001**,#	0.292#
	Bleeding per rectum	11 (55.0%)	11 (55.0%)	11 (55.0%)	1.000#	1.000#	1.000#
	Diarrhea	0 (0.0%)	5 (25.0%)	6 (30.0%)	*0.017**#	*0.008**#	0.723#
	Constipation	14 (70.0%)	8 (40.0%)	8 (40.0%)	0.057#	0.057#	1.000#
	Signs						
	Anemic manifestations	17 (85.0%)	13 (65.0%)	15 (75.0%)	0.144#	0.429#	0.490#
	Cachexia	9 (45.0%)	0 (0.0%)	0 (0.0%)	*< 0.001**#	*< 0.001**#	1.000#

Ctrl control, *DM* diabetes mellitus, *HTN* hypertension, *SD* standard deviation

^ Two-tailed Student's *t* test

Chi-square test

*Significant at $p < 0.05$

Fecal Tumor M2PK Concentrations

All patients received a toilet hat for stool collection and were instructed to collect a single walnut-size stool sample 1 day before the laxative administration in preparation for colonoscopy. No special diet was recommended before giving the sample. The pre-colonoscopy stool samples were stored at − 20 °C until later use. Fecal tumor M2PK concentrations were determined in homogenized stool samples using a sandwich ELISA method designed to specifically recognize the dimeric form of M2PK (ScheBo® Biotech AG, Giessen, Germany). A positive test result was defined as > 4 kU/L.

Statistical Analysis

Data was statistically analyzed using the Statistical Package for Social Sciences (SPSS) software version 22, USA, 2013. Quantitative data were presented as minimum and maximum of the range. Mean ± SD (standard deviation) was calculated for quantitative parametric data, median and first and third interquartile range for quantitative nonparametric data, while qualitative data were presented as number and percentage.

Independent *t* test was used to analyze variance between independent groups with parametric data, and the Mann Whitney *U* in case of two independent groups with nonparametric data. Chi-square test was used for differences between proportions. Receiver operating characteristic (ROC) curve was used to evaluate the performance of multivariable that differentiate between certain groups. Significance was at alpha (P) < 0.05.

Table 2 Tumor markers in smokers compared with nonsmoker subjects

Group	Smoking		Nonsmoking		*P*
	N	Median (IQR)	*N*	Median (IQR)	
CRC group					
CEA	11	10.0 (2.4 – 100.0)	9	37.0 (4.1 – 79.0)	0.939
CA19-9	11	233.0 (16.0 – 665.0)	9	30.0 (14.4 – 586.0)	0.790
M2PK	11	58.2 (13.2 – 92.4)	9	82.9 (18.6 – 99.2)	0.732
CRP group					
CEA	10	3.0 (0.9 – 8.6)	10	2.1 (0.9 – 4.4)	0.733
CA19-9	10	19.1 (10.3 – 24.8)	10	14.1 (4.7 – 20.0)	0.226
M2PK	10	3.3 (1.2 – 11.6)	10	8.3 (4.6 – 13.4)	0.131
Control group					
CEA	7	2.0 (0.8 – 5.0)	13	2.0 (1.0 – 4.0)	0.780
CA19-9	7	10.0 (7.0 – 13.0)	13	4.0 (2.2 – 12.5)	0.068
M2PK	7	3.8 (3.0 – 9.7)	13	2.8 (0.0 – 4.1)	0.095

Table 3 Per-rectum examination findings among the studied groups

		CRC ($N=20$)	CRP ($N=20$)	Control ($N=20$)	CRC/CRP[#]	CRC/Ctrl[#]	CRP/Ctrl[#]
Normal		13 (65.0%)	19 (95.0%)	12 (60.0%)	*0.030**	*0.007**	0.598
Piles		0 (0.0%)	0 (0.0%)	8 (40.0%)			
Rectal mass		7 (35.0%)	1 (5.0%)	0 (0.0%)			
ESR (ml/h)	Mean ± SD	74.5 ± 21.0	42.1 ± 10.0	40.3 ± 15.3	*<0.001*[*,^]	*<0.001*[*,^]	0.662[^]
	Range	20.0–110.0	30.0–60.0	10.0–75.0			
M < 20							
F < 30							

[#] Chi-square test

[^] Independent *t* test

*Significant

Results

No Significant Variability Between the Studied Groups Concerning Age, Gender and Associated Past Medical Comorbidity and Family History

Patients aged 26 to 52 were recruited to the study. Apart from two patients with family history of CRC, there was no remarkable past medical or family history among the groups, Table 1. Although smoking was noted to be higher in CRC and CRP, no significant difference was identified in comparison to healthy subjects, Table 2.

Patients with CRC Present Mainly with Cachexia and Weight Loss

Although weight loss and anemia are the most clinically valuable diagnostic tools for CRC, in our study, only weight loss and cachexia were significantly higher among CRC patients ($P = 0.001$). This could be attributed to that our clinics mainly serve patients with low socioeconomic status. These patients usually present with rectal bleeding due to infectious diarrhea. Thus, diarrhea was significantly higher ($P = 0.017$) in the non-CRC groups, Table 1.

Table 4 Tumor markers among the studied groups

Measures		CRC (N=20)	CRP (N=20)	Control (N=20)	CRC/CRP	CRC/Ctrl	CRP/Ctrl
CEA (ng/mL)	Median (IQR)	23.5 (3.6–85.8)	2.3 (0.9–4.7)	2.0 (0.9–4.0)	*< 0.001*[*,^]	*< 0.001*[*,^]	0.755[^]
	Range	0.9–100.0	0.6–23.0	0.5–5.2			
	Smoker > 5/ Nonsmoker > 10	10 (50.0%)	3 (15.0%)	1 (5.0%)	*0.018*[*,#]	*< 0.001*[*,#]	0.292[#]
CA19-9 (ng/mL)	Median (IQR)	134.5 (16.8–613)	16.9 (6.4–22.3)	6.0 (3.0–12.8)	*0.004*[*,^]	*< 0.001*[*,^]	*0.005*[*,^]
	Range	3.6–892.0	0.2–71.0	0.0–23.0			
	> 30.9	11 (55.0%)	2 (10.0%)	0 (0.0%)	*0.002*[*,#]	*< 0.001*[*,#]	0.147[#]
M2PK (u/mL)	Median (IQR)	70.0 (16.0–92.8)	6.6 (2.7–11.5)	3.1 (1.9–4.4)	*< 0.001*[*,^]	*< 0.001*[*,^]	*0.022*[*,^]
	Range	2.8–189.7	0.5–23.7	0.0–10.8			
	> 4.0	17 (85.0%)	12 (60.0%)	5 (25.0%)	0.077[#]	*< 0.001*[*,#]	*0.025*[*,#]
FOBT		12 (60.0%)	12 (60.0%)	14 (70.0%)	1.000[#]	0.507[#]	0.507[#]

IQR interquartile range

[^] Mann Whitney test

[#] Chi-square test

*Significant at $P < 0.05$

Table 5 Diagnostic characteristics of tumor markers cutoff points in differentiating CRC group from CRP group

Marker	Measure	DA	Sen.	Sp.	PPV	NPV	LR+	LR−	LR
CEA Smoker > 5/ N > 10	Value	67.5	50.0	85.0	76.9	63.0	3.3	0.6	5.7
	95% CI	50.5–78.2	33.0–60.7	68.0–95.7	50.7–93.4	50.4–70.9	1.0–14.1	0.4–1.0	1.0–34.4
CEA > 25.0	Value	75.0	50.0	100.0	100.0	66.7	> 100	0.5	> 100
	95% CI	60.0–75.0	35.0–50.0	85.0–100.0	69.9–100.0	56.6–66.7	2.3– > 100	0.5–0.8	3.0– > 100
CA19-9 > 30.9	Value	72.5	55.0	90.0	90.0	66.7	5.5	0.5	11.0
	95% CI	55.7–80.7	38.2–63.2	37.2–98.2	73.2–98.2	54.2–72.7	1.4–34.4	0.4–0.08	1.7–91.7
CA19-9 > 75.0	Value	70.0	50.0	90.0	83.3	64.3	5.0	0.6	9.0
	95% CI	53.4–78.2	33.4–58.2	73.4–98.2	55.6–96.6	52.4–70.1	1.3–31.7	0.4–0.9	1.4–74.6
M2PK > 4.0	Value	62.5	85.0	40.0	58.6	72.7	1.4	0.4	3.8
	95% CI	46.3–73.2	68.8–95.7	23.8–50.7	47.4–66.0	43.2–92.2	0.9–1.9	0.1–1.3	0.7–23.0
M2PK > 25.0	Value	87.5	75.0	100.0	100.0	80.0	> 100	0.3	> 100
	95% CI	72.3–87.5	59.8–75.0	84.8–100.0	79.7–100.0	67.8–80.0	3.9– > 100	0.3–0.5	8.3– > 100

CI confidence interval, *DA* diagnostic accuracy, *Sen* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR−* negative likelihood ratio

BPR Findings Among the Studied Groups

Bleeding Per-rectum examination (BPR) revealed masses in 35% of CRC patients and 5% of CRP patients and none in the control group. ESR was significantly higher among CRC group (74.5 ± 21.0, range 20.0–110.0) compared to CRP (42.1 ± 10.0, range 30.0–60.0) and the control groups (40.3 ± 15.3, range 10.0–75.0), $P < 0.001$. Table 3.

Tumor Markers Are Significantly Higher in All CRC Patients

CEA was significantly higher among CRC group compared to CRP and the control groups. CA19-9 and M2PK differed significantly between all groups, the highest among CRC group, followed by CRP, and least among the control group Table 4. Diagnostic characteristics of tumor marker cutoff points in differentiating CRC group from CRP and CRC from control are presented in Tables 5 and 6, respectively. Diagnostic performance of tumor markers in differentiating the studied groups presented as the area under the curve (AUC) is presented in Table 7.

Pelvic-Abdominal US, CT, Colonoscopy and Histopathology Examination

Twenty-five percent of CRC patients were found to have focal liver lesions when examined by abdominal

Table 6 Diagnostic characteristics of tumor marker cutoff points in differentiating CRC group from control group

Marker	Measure	DA	Sen.	Sp.	PPV	NPV	LR+	LR−	LR
CEA Smoker > 5.0/NS > 10.0	Value	72.5	50.0	95.0	90.9	65.5	10.0	0.5	19.0
	95% CI	56.5–77.2	34.0–54.7	79.0–99.7	61.7–99.5	54.5–68.8	1.6–207.5	0.5–0.8	1.9–457.2
CEA > 5.5	Value	77.5	55.0	100.0	100.0	69.0	> 100.0	0.5	> 100.0
	95% CI	62.3–77.5	39.8–55.0	84.8–100.0	72.4–100.0	58.5–69.0	2.6– > 100.0	0.5–0.7	3.7– > 100.0
CA19-9 > 30.9	Value	77.5	55.0	100.0	100.0	69.0	> 100.0	0.5	> 100.0
	95% CI	62.3–77.5	39.8–55.0	84.8–100.0	72.4–100.0	58.5–69.0	2.6– > 100.0	0.5–0.7	3.7– > 100.0
CA19-9 > 25.0	Value	82.5	65.0	100.0	100.0	74.0	> 100.0	0.4	> 100.0
	95% CI	67.2–82.5	49.7–65.0	84.7–100.0	76.5–100.0	62.7–62.7	3.2– > 100.0	0.4–0.6	5.5– > 100.0
M2PK > 4.0	Value	80.0	85.0	75.0	77.3	83.3	3.4	0.2	17.0
	95% CI	62.6–90.4	67.6–95.4	57.6–85.4	61.5–86.7	64.0–94.9	1.6–6.5	0.1–0.6	2.8–121.2
M2PK > 12.0	Value	90.0	80.0	100.0	100.0	83.3	> 100.0	0.2	> 100.0
	95% CI	75.0–90.0	65.0–80.0	85.0–100.0	81.2–100.0	70.8–83.3	4.3– > 100.0	0.2–4.0	10.5– > 100.0

CI confidence interval, *DA* diagnostic accuracy, *Sen* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR−* negative likelihood ratio

Table 7 Diagnostic performance of tumor markers in differentiating the studied groups

Variables	AUC	SE	*P*	95% CI
CRC from CRP				
CEA	0.794	0.070	< *0.001**	0.656–0.932
CA19-9	0.763	0.079	*0.005**	0.608–0.917
M2PK	0.866	0.061	< *0.001**	0.746–0.987
CRC from control				
CEA	0.825	0.066	< *0.001**	0.696–0.954
CA19-9	0.888	0.053	< *0.001**	0.784–0.991
M2PK	0.925	0.043	< *0.001**	0.826–1.000
CRP from control				
CEA	0.529	0.093	0.756	0.346–0.711
CA19-9	0.759	0.078	*0.005**	0.607–0.911
M2PK	0.711	0.083	*0.022**	0.548–0.875

AUC the area under the curve, *SE* standard error, *CI* confidence interval
*Significant at $P < 0.05$

US or computed tomography (CT) scan. These lesions were undetectable in CRP and the control groups.

Colonoscopy exams were highly sensitive in detecting rectal and colonic masses and polyps. All CRC patients had either rectal or colonic masses, all CRP patients had polyps, and 60% of the control group had nonspecific findings including 40% with internal piles. Infiltrating carcinoma was identified in 100% of biopsies from CRC patients. CRP were histologically classified into hyperplastic polyp (30%), had tubulovillous adenoma with no dysplasia (5%), tubulovillous adenoma with low-grade dysplasia (55%), and tubulovillous adenoma with high-grade dysplasia (10%). On the other hand, 90% of the control group had nonspecific colitis and 10% were normal.

Tumor Markers Cutoff Points in Differentiating CRC Group from CRP Group

Table 5 presented that M2PK > 25 was significantly higher in the CRC group compared to CRP group. M2PK > 12, as shown in (Table 6), had the highest characteristics in differentiating CRC group from the control group, had perfect specificity and high sensitivity. Figure 1 showed significant positive correlations between the tumor markers CEA and CA19-9 among CRC and CRP groups.

Diagnostic Performance of Tumor Markers in Differentiating the Studied Groups Using ROC Analysis

M2PK revealed good diagnostic performance as shown in (Tables 5 and 6 and Fig. 2a–c) in differentiating CRC group from CRP group, but excellent diagnostic performance in differentiating CRC group from the control group. CA19-9 and CEA revealed poor diagnostic performance in differentiating CRC-group from CRP group, while the combined CA19-9 and CEA improved the diagnostic performance. Fig. 2a–c.

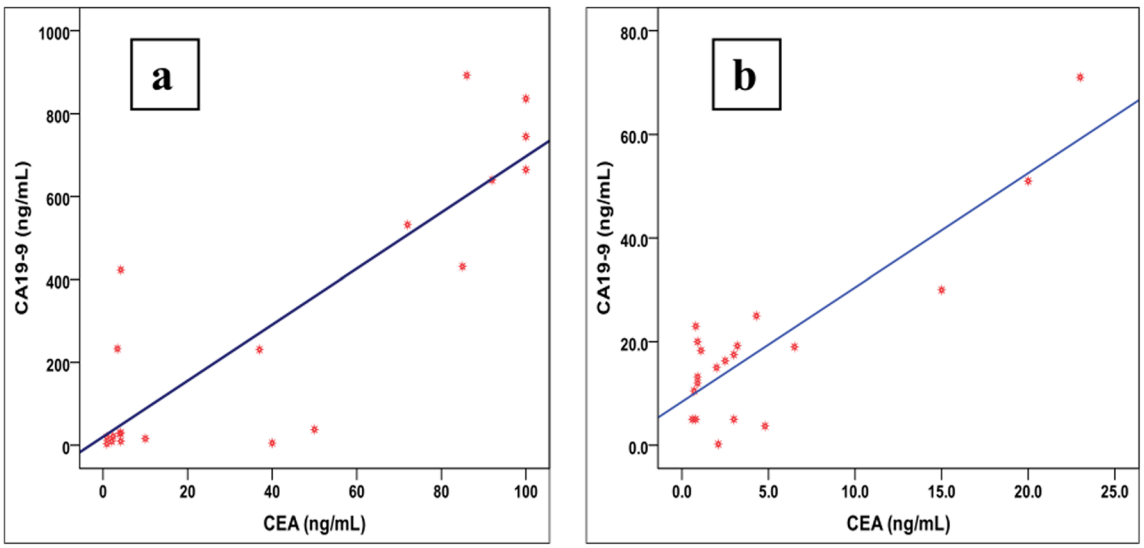

Fig. 1 Correlation between CEA and CA19-9 among the CRC and CRP groups. **a** Correlation between CEA and CA19-9 among the CRC group. **b** Correlation between CEA and CA19-9 among the CRP group

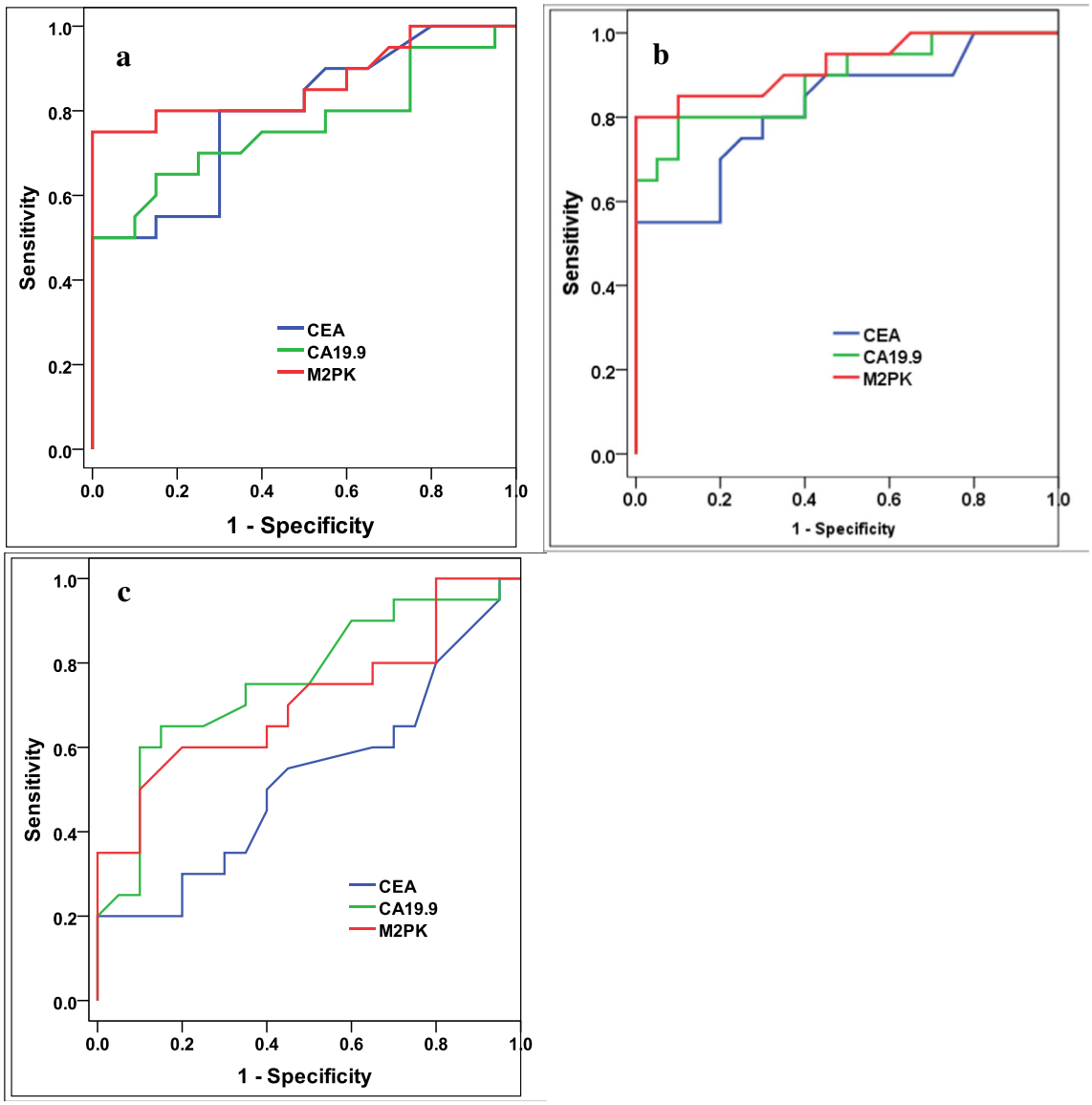

Fig. 2 Diagnostic performance of tumor markers in differentiating (**a**) CRC from CRP group (**b**), CRC from the control group, and (**c**) CRP from the control group

The Diagnostic Characteristics of Serial Application of M2PK > 25 Followed by CA19-9 > 25 Cutoff Points in Differentiating CRC Group from CRP and Control Groups

A subject can be diagnosed as CRC if he/she has M2PK > 25, or has M2PK ≤ 25 and CA19-9 > 25; this serial test has a better specificity and sensitivity, as presented in Tables 5 and 6.

Discussion

Our aim is to search for sensitive and specific noninvasive screening tool for early detection and treatment of CRC patients. According to our data, M2PK can be of value in differentiating CRC patients from those with CRP or the healthy subjects. Patients with M2PK > 25 or M2PK ≤ 25 and CA19-9 > 25 have a higher probability of CRC.

Bleeding per rectum was the most common presenting complaint (55%) in all groups. This was associated with weight loss and cachexia in patients with CRC. Although no significant differences were identified in the prevalence of anemia and constipation among all groups, it was more prevalent in our CRC patients, similarly to Fletcher's studies [17]. On the other hand, comorbidity of diarrhea and rectal bleeding was the main presenting symptom in CRP and control groups. Infectious diarrhea is a common presenting disease in our tropical clinics that usually presents with bleeding per rectum, hence the prevalence of the later symptom in all groups. As expected, cachexia was significantly

higher only in our CRC patients, consistently with Fletcher [17].

We evaluated the diagnostic power of all tumor markers in differentiating between CRC and CRP and between CRC and healthy group. Sensitivity, specificity, negative and positive predictive values (NPV, PPV), as well as positive and negative likelihood ratios (PLR and NLR), were statistically calculated for all tumor markers. In this study, CEA, CA19-9, and M2PK tumor markers were significantly higher in CRC group compared to that in CRP and the control groups, ($P < 0.001$). Also, we identified M2PK with a cutoff > 25 as the test with the highest diagnostic accuracy. In contrast to other tests, M2PK has a DA of 87.5%, sensitivity of 75%, and specificity of 100%. This data is consistent with Möslein et al. [18] who reported specificity of fecal M2PK of 89.5%. Similarly, Kim et al. [4] reported that sensitivity of fecal M2PK was 92% in CRC and was 69% in CRP with specificity of 83%.

Our study revealed that CEA had 50% sensitivity and 90% specificity in the diagnosis of CRC. Researchers have reported different sensitivity and specificity values for CEA as a tumor marker. Kim et al. [19] reported that CEA has 22% sensitivity in CRC and 100% specificity. Similarly, CA19-9 had sensitivity 55% and specificity 90% in CRC group, and Wang et al. [20] reported that sensitivity of CA19-9 in CRC is 69% and specificity is 61%. Although increasing the cutoff above 25 increased the DA and sensitivity of M2PK, no improvement occurred in the DA and sensitivity of CEA and CA19-9 in our patients. Table 4 shows that FOBT levels scored insignificant differences among the studied groups, consistently with others [21].

Radiologically, 25% of our CRC group presented with focal lesions in the liver that were absent in CRP and control groups. These focal lesions were metastases from primary tumors, which were diagnosed by triphasic CT for those patients. In addition to the radiological findings, the histopathological examination of the colonoscopic findings confirmed the diagnosis and found that all CRC patients had infiltrating adenocarcinoma. Sithambaram et al. [22] reported that 30% of patients with CRC have liver metastasis at diagnosis and also reported no significant differences concerning the sensitivity, specificity, positive and negative predictive values, and accuracy of the M2PK test for the different tumor stages. In our study, we did not observe histopathological M2PK differences among the different classes of CRP (data not shown).

Our patients with intramucosal carcinoma or carcinoma in situ were classified as having high-grade dysplasia. Histological classification of total polyps included adenoma (tubular, tubulovillous, or villous) and non-adenomatous polyps. Bond et al. [23] reported that fecal M2PK revealed an increasing positive frequency as the pathology progressed along the adenoma-carcinoma pathway, supporting potential utility for the assay to distinguish between absence and presence of neoplastic disease [24].

A variety of tumor markers have been shown to increase in CRC. The investigated markers in our study, CEA and CA19-9, were significantly higher in CRC in comparison to that in CRP and control groups. Although CA19-9 was significantly higher in CRP compared to that in the controls, no significant differences were found between CRP and the control regarding CEA. We found a significant positive correlation between CEA and CA19-9 among CRC and CRP groups, consistently with others [25]. Despite the significant correlation, CEA and CA 19-1 have a lower sensitivity compared to other screening tests measured in this study. Also, there is lack of evidence that CEA monitoring adds value to the patients' quality of life and mortality [26].

Concerning the diagnostic performance of tumor markers in differentiating the study groups, M2PK has a good diagnostic performance in differentiating CRC from both CRP and control groups. Thus, although M2PK is superior to other tests, all markers had poor diagnostic performance in differentiating CRP from the control group, Fig. 2c.

Overall, early diagnosis of CRC patients is of utmost importance, and screening with various non-invasive tests can be live-saving. In the current study, we validated the role of M2PK as a sensitive marker for early detection of CRC in Egyptian patients. In addition, only weight loss and cachexia were the most differentiating clinical symptoms and signs for CRC. For future research, we recommend (1) clinical studies to better assess the use of fecal M2PK as a routine in vitro diagnostic test for CRC screening, (2) multicenter studies with larger sample size to assess the value of fecal M2PK in screening CRP, (3) further studies to compare fecal M2PK and other markers (e.g., fecal DNA and fecal matrix-metalloproteinase-9) in CRC screening, and (4) longitudinal follow-up of the prognostic value of fecal M2PK post therapy to assess the survival rate.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study ethics protocol was approved by the Tropical Medicine Department, Ain-Shams University and the Gastroenterology and Hepatology Department, Theodor Bilharz Research Institute, Cairo, Egypt.

Consent to participate Written consent to participate was given by all patients who were enrolled in the study.

Abbreviations *ANOVA*, analysis of variance; *BPR*, bleeding per rectum; *CA 19-9*, carbohydrate antigen 19-9; *CEA*, carcinoma embryonic antigen; *CRP*, colorectal (adenomatous) polyps; *CRC*, colorectal cancer; *CT*, computerized tomography; *Ctrl*, control; *DA*, diagnostic accuracy; *DM*, diabetes mellitus; *ELISA*, enzyme-linked immunosorbent assay; *ESR*, erythrocyte sedimentation rate; *FOBT*, fecal occult blood test; *HTN*, hypertension; *INR*, international normalized ratio; *LR*, likelihood ratio; *NCI*, National Cancer Institute; *NHL*, non-Hodgkin lymphoma; *NPV*, negative predictive value; *PTT*, partial thromboplastin time; *PT*, prothrombin time; *PPV*, positive predictive value; *M2PK*, pyruvate kinase isoenzyme type M2; *ROC*, receiver operating characteristic; *SPSS*, Statistical Package for Social Sciences

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