Fecal Tumor M2-Pyruvate Kinase ™ (ScheBo Test) for Colorectal Cancer Detection in Adult Egyptians

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Abstract

Introduction: *Colorectal cancer (CRC) represents the 3rd most common tumor worldwide and the 6th in Egypt. It is still burdened by significant morbidity and mortality despite several therapeutic improvements. A gold standard for early detection and non invasive diagnosis is lacking. Aim was to assess faecal tumor M2-PK™ (ScheBo test) for detection of CRC.ln Adult Egyptians*

Methods: *This cross-sectional, case-control study was carried out on 66 subjects. The cases group comprised 46 consecutive treatment- naïve patients with sporadic CRC proved by colonoscopy, histopathology and abdominal computed tomography which all together helped in tumor staging with TNM and Duke's staging systems. Twenty apparently healthy subjects (without CRC and with normal colonoscopy) served as the control group (age and sex matching the cases group). Faecal tumor M2-PK™ (ScheBo test) was assessed in all the study subjects.*

Results: *Cases were 24 males and 22 females with a mean age of 50.6 y$_s$ and the range was 22-81y$_s$. Age ditribution showed 12 cases (26%) below 40 and 50% above 50 y$_s$. Bleeding per rectum was the main presenting symptom (39%) followed by recent onset constipation (34.8%) in the studied cases. Rectal lesions were found during colonoscopy in 47.8% of cases while colonic lesions were found in 52.2%. Mass was the most encountered gross pathology (in 67.4%, 31 cases) followed by ulcer (in 28.3%, 13 cases) and stricture (in 4.3%, 2 cases). Adenocarcinoma was the commonest type (76.1 %, 35 cases) followed by mucinous adenocarcinoma (15.2 %, 7 cases). The fecal levels of tumor M2-PK were significantly higher (P<0.001) in cases compared to the control (35.66 ± 17.32 and 2.74 ± 4.36 U/ml, respectively). Fecal tumor M2-PK levels showed a significant positive correlation with both TNM and Duke's stages (p<0.05), while no significant correlation was found with age and sex. Applying ROC curve, at a cut-off value of 3.9 U/ml, fecal Tumor M2-PK was 97.8% sensitive and 85% specific for detection of CRC with AUROC = 0.96.*

Conclusion: *Fecal tumor M2-PK was a sensitive and specific marker for detection of CRC at values ≥ 3.9 U/ml and positively correlated with the tumour grade and stage.*

Key Words*: colorectal cancer, ScheBo Test, M2-PK*

I. Introduction

Colorectal cancer (CRC) represents the third most common tumor worldwide, and is still burdened by significant morbidity and mortality despite several therapeutic improvements [1]. CRC incidence rates are rapidly increasing due to the effect of many risk factors, including smoking, physical inactivity, obesity, red and processed meat consumption, as well as excessive alcohol intake [2]

In Egypt, CRC is one of the most common malignant neoplasms. Its incidence ranges between 2-6 % of the total number of cancer cases reported annually and it ranks as the sixth most common cancer in both males and females [3]. The median age of CRC cases in Egypt is 48 years for both males and females [4].

Most cases of CRC develop from polyps. Three types of polyps occur: hamartoma (junior polyp), hyperplastic mucosal proliferation (hyperplastic polyp) and adenomatous polyp. Adenomatous is the only premalignant type, it may be tubular, villous, or tubulovillous, with villous adenomas the most likely to be cancerous. Adenomatous polyps may be either sessile (flat) or pedunculated (stalked), with sessile types being more likely to progress to cancer. Finally, polyps greater than 2.5 cm are five times more likely to be cancerous than those less than 1.5 cm. Overall, once an adenomatous polyp forms, it takes at least 5 years of growth to

reach clinical significance, suggesting the need to initiate screening and perform routine follow-up evaluation to identify polyps that are of concern before they become cancerous [5].

CRC mortality rates can be decreased by early diagnosis through screening. However, the present CRC screening techniques (colonoscopy, fecal occult blood test (FOBT), and serum carcinoembryonic antigen (CEA) testing are limited by their difficulties and costs beside uncertain or delayed results [6]. Hence, the identification of biomarkers that are simple, noninvasive, cost-efficient and reasonably sensitive/specific is urgently needed [7].

M2-PK is a key enzyme within glycolysis, a process that catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate. Depending upon the metabolic functions of the tissues, different isoenzymes of pyruvate kinase are expressed. During tumour formation, the tissue-specific isoenzymes disappear and the pyruvate kinase isoenzyme type M2 is expressed [8].

The *aim* of the present study was to assess faecal tumor M2-PK™ (ScheBo test) as a marker for CRC detection in Adult Egyptian patients

II. Patients and methods

This cross-sectional, case-control study was carried out on 66 subjects. The cases group comprised 46 consecutive patients with CRC who were attending the Departments of Hepatology, Gastroenterology and Infectious Diseases and Internal medicine at Benha University Hospital and Surgical Oncology at National Cancer Institute -Cairo, within the period between January 2015 and March 2016. Another 20 apparently healthy subjects (without CRC and with normal colonoscopy) served as the control group (age and sex matching the cases group). The indication for colonoscopy in these subjects was either long history of unexplained abdominal pain and/or altered bowel habits.

The study protocol was approved by the Ethical Committee of Benha Faculty of Medicine, Benha University. Patients with the following criteria were excluded from the study namely; those refusing the written medical consent, pregnant females, patients with familial adenomatous polyposis or hereditary nonpolyposis CRC, those who were undergoing or received chemotherapy and/or radiotherapy, those with inflammatory bowel diseases, primary sclerosing cholangitis and other malignancies. All the studied cases had given an informed written medical consent. They were subjected to thorough history taking, general and abdominal examination as well as per rectal examination, laboratory investigations; namely: stool analysis, complete blood count (CBC), erythrocyte sedimentation rate (ESR), random blood suger (RBS), glycated hemoglobin (Hb A1C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum albumin, serum bilirubin (total and direct), prothrombin time (PT) and concentration, serum creatinine and blood urea, carcino-embryonic antigen (CEA) and CA 19-9. Sandwich ELISA with two monoclonal antibodies highly specific for human tumour M2-PK (ScheBo®, Biotech Aktiengesellschaft, Germany) was applied for quantitative assessment of fecal tumor M2-PK™.

Abdomino-pelvic ultrasonography, computed tomography (CT scan) and complete colonoscopy followed by histopathological examination of the biopsied specimens.
Finally, TNM- and Duke's staging systems were applied.

Statistical analysis: was done using SPSS 20, Quantitative data was expressed in Mean ± Standard Deviation, Qualitative data was expressed in number and percentage. Comparison between groups was done using Mann-Whitney U and Kruss-Kal Wallis test, The sensitivity and specificity were examined at different cutoff points using ROC curve analysis to determine the best cutoff point as well as the diagnostic power of each test.   A P value <0.05 was considered statistically significant (S).

III. Results

This study evaluated 46 patients with CRC (24 males and 22 females) with mean age of 50.6 y_s and range 22-81y_s. The 20 healthy controls were 13 males and 7 females with mean age of 54.1 y_s and range of 27-80 y_s. The mean age of male patients (50.3 y_s) was relatively (non significantly) younger than that of female patients (51.1y_s). Distribution of age among the studied cases showed 26% below 40 y_s and 50% above 50 y_s.
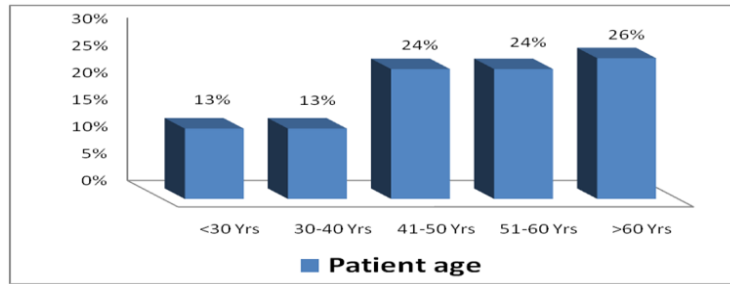
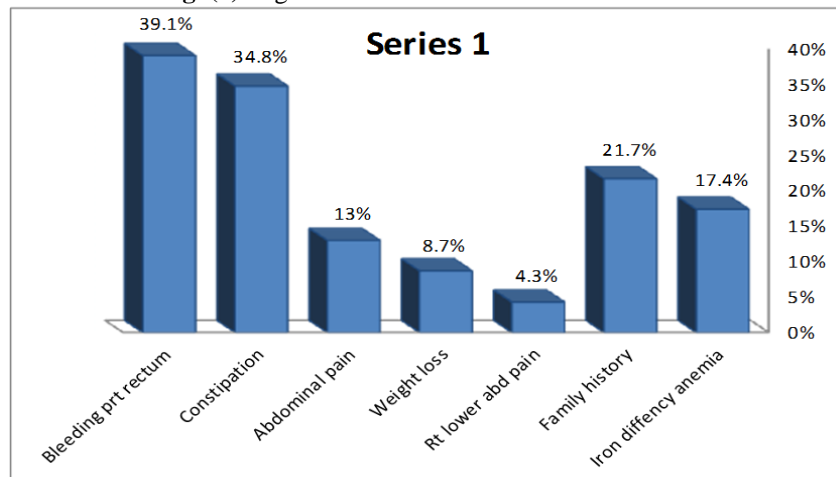**Fig. (1):** Age distribution within the studied cases.

Fig. (2): Presenting symptoms and history findings among the studied cases.

Bleeding per rectum was the main presenting symptom followed by recent onst constipation in the studied cases. Positive family history (1st or 2nd degree relatives) was found in 21.7% of the studied cases while anemia leading to blood transfusion was found in 17.4% of them.
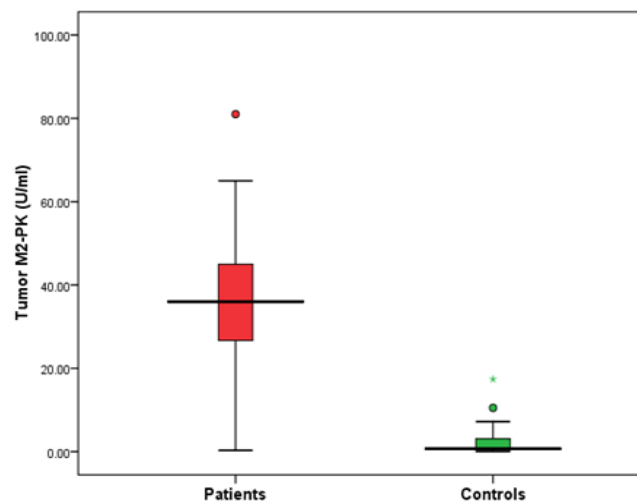

Fig. (3): Fecal Tumor M2-PK level in the studied groups.

The fecal levels of tumor M2-PK were significantly higher (P<0.001) in the studied cases compared to the control group (35.66 ± 17.32 and 2.74 ± 4.36 U/ml respectively). The median fecal tumor M2-PK level in the control group was 0.7 U/ml and ranged from 0.02 U/ml to 17.4 U/ml. The median fecal tumor M2-PK level in cases was 36 U/ml and ranged from 0.3 U/ml to 81U/ml.

**Table (1):** Fecal M2PK in Diabetes and Smoking.

		No	Mean	S.D	Mann-Whitney U	p-value

|						
Diabetes	Diabetic	22	29.06	16.44	2.6	0.01
	Non- diabetic	24	41.13	16.69		
Smoking	Smokers	26	34.79	20.04	0.5	0.6
	Non-smokers	20	37.09	13.15		

There was a statistically significant difference in fecal M2PK level between diabetics which is significantly lower (29.06± 16.44 U/ml) than nondiabetic (41.13 ± 16.69 U/ml) (p= 0.01) while a non significant difference was found between smokers (34.79 ± 20.04 U/ml) and non-smokers (37.09± 13.15 U/ml) (p-value 0.6).

Fig (4): Correlation between fecal M2PK and BMI
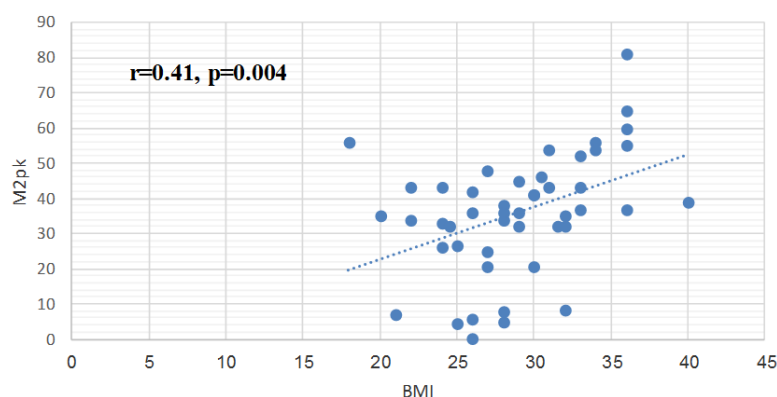
There was a statistically significant positive correlation between fecal M2PK level and BMI in the studied cases (p-value = 0.004) .
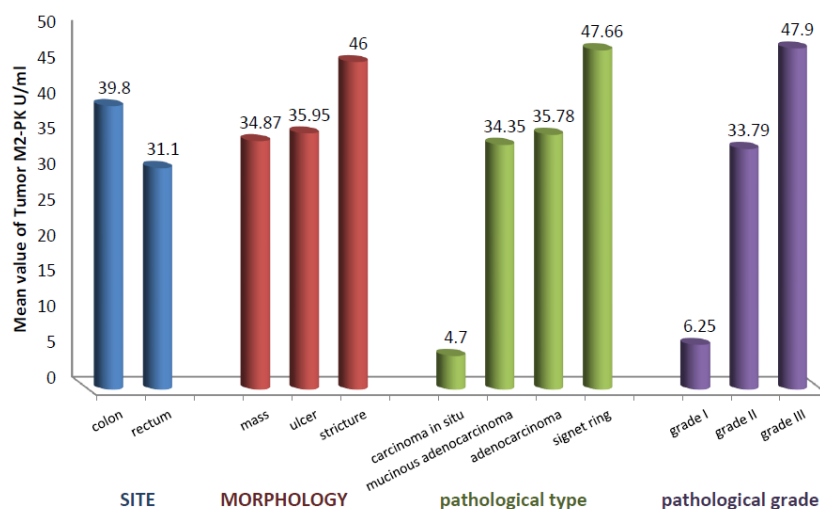

Fig. (5): Mean value of Fecal Tumor M2-PK according to colonoscopic and histopathological findings.

There was no statistically significant difference in fecal Tumor M2-PK levels as regards site, morphology and pathological types of CRC, while there was a statistically significant rise in fecal Tumor M2-PK levels as regards pathological grades. The mean value of grade I was (6.25 U/ml), grade II (33.79 U/ml) and grade III (47.9U/ml) (P<0.05). The higher the pathological grade of CRC the higher the mean value of Tumor M2-PK.

Table (2) and Fig (6) : Fecal Tumor M2-PK levels in different TNM and Dukes stages.

Variable		Nº	Tumor M2-PK (U/ml)		KWT test	P	Sig pairs
			Mean ± SD	Range			
T stage	Tis/ T1	3	11.80 ±10.88	4.7- 5.1	10.2	0.017 (S)	Tis/T1≠T3 Tis/T1≠T4
	T2	18	31.87 ±13.75	4.5-54			
	T3	23	40.23 ±17.68	0.3-81			
	T4	2	53.00 ±9.89	46-60			
N stage	N0	26	30.40 ±14.03	0.3-54	10.7	0.006 (S)	N0≠N2
	N1	11	38.80 ±15.73	4.5-65			
	N2	8	52.32 ±17.30	20.6-81			
Duke A		3	12.70 ±10.88	4.7-25.1	7.15	0.028 (S)	A≠B A≠C
Duke B		24	33.33 ±15.47	0.3-60			
Duke C		19	42.23 ±17.03	4.5-81			
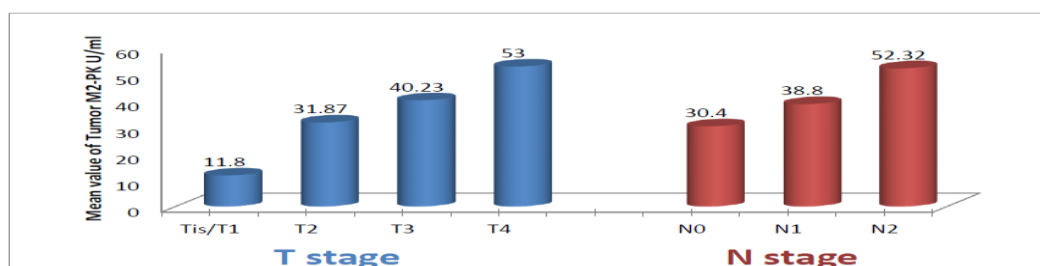
There was a statistically significant difference in fecal Tumor M2-PK levels in different TNM Stages. There was also a significant difference in fecal Tumor M2-PK levels in different Dukes stages. (P <0.05).

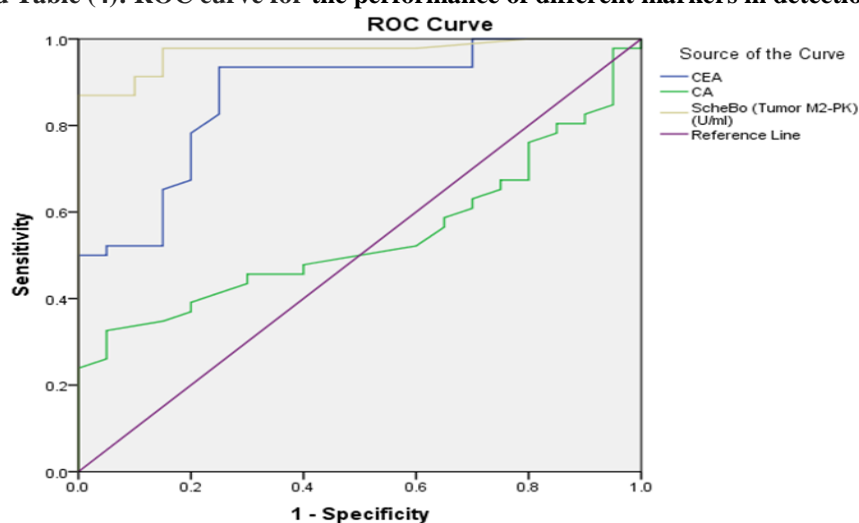**Table (3) and Fig. (7): ROC curve for the performance of Tumor M2-PK in detection of CRC.**

Tumor M2-PK U/ml	Sens%	Spec%	PPV%	NPV%	AUC	95%CI	P
≥3.9	97.8%	85%	93.8%	94.4%	0.96	0.92-1.0	<0.001 (HS)
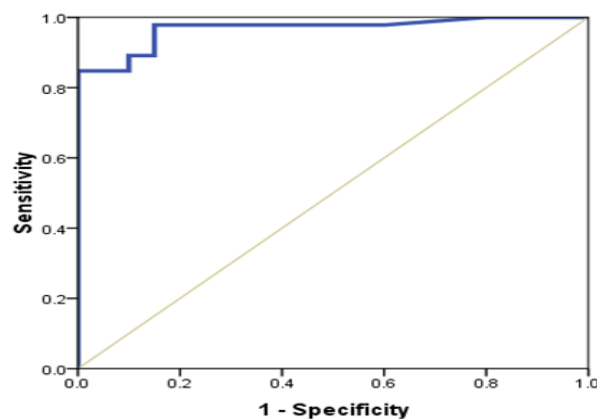
At a cut-off value of 3.9 U/ml, fecal Tumor M2-PK was 97.8% sensitive and 85% specific for detection of CRC with AUROC = 0.96.

Fig (8) and Table (4): ROC curve for the performance of different markers in detection of CRC.

Tumour markers	AUROC
CEA	0.871
CA19-9	0.543
ScheBo (Tumor M2-PK) (U/ml)	**0.971**

ROC curve reveals that Tumor M2-PK was better than CEA and CA 19.9 in detection of CRC at any stage.

IV. Discussion

In the present study, the age of the studied cases ranged between 22-81ys, with a mean of 50.6 ± 15.1ys. Fifty percent were > 50ys and 26% (more than 1/4 of patients) were ≤ 40ys **.** This comes in agreement with [9,10] who found that the mean age of their assessed Egyptian patients was 51ys and 44.8ys respectively. *El Attar, (2005) and Sakr et al., (2016)* told that the median age of CRC patients in Egypt was 48 and 51.2 ys respectively [5,10]. *Basu et al., (2016) and Nataraj et al., (2016)* found the mean age of their assessed Indian patients was 52.8 ± 13.5 and 49.31 ys, respectively[11,12]. On the other hand; [13] found that the mean age of their assessed American Non-Hispanic white and American Asian patients was 70.3ys and 66.3ys respectively.[13] while [14] found that the median age of CRC cases was 70 years in Germany[14].

About 1/4 of the assessed cases in the current study were below the age of 40 ys. This comes in agreement with [9,15,16,17] who found that CRC cases under the age of 40ys were 35.6 %, 25 %, 22 %, 38 % and one third respectively in their studied Egyptian patients. In Asia, [18] told that 19.5-28.6 % of patients were less than 40ys and in Mexico, National Cancer Institute reported 22.8%. On the contrary, [19] in USA, mentioned that CRC is rare before age 40ys in both men and women. [20]) told that CRC is rare before the age of 40 except for those with genetic predisposition or predisposing conditions and mentioned that the frequency of CRC in patients less than 40 ys in USA, France, Australia and Denmark was 6-7 %, 3.1 %, 5 % and 2.5 % respectively. The difference in mean ages could be attributed to different risk factors, dietary patterns, life style and life expectancy in different cultures and societies. This finding may enhance the need for early screening for CRC among the Egyptian population**.**[18]

Males represented 52.2% (and females, 47.8%) of the studied cases in the present study, with no statistically significant difference (p > 0.05)**.** This comes in agreement with [10,21,22,23] who told that CRC affects men and women almost equally. In disagreement with this, [24,25] told that men have more incidence of CRC than women. The percentage of the studied cases with CRC with a positive family history was 21.7 %. This comes in agreement with [26,27] who told a percentage of up to 20 %. [28] reported a higher figure (31.3 %) in Iranian patients. These data make adherence to CRC screening programs mandatory in subjects with family history of CRC.

In the present study, bleeding per rectum was the commonest presenting symptom (39.1 %) followed by recent onset constipation (34.8 %) which is concomitant with the local reports of [29]) who met bleeding per rectum as the main presenting symptom of their studied cases.

Mass was the commonest gross pathology (67.4 %) detected in the studied cases followed by ulcer 28.3 % and stricture 4.3 %. This comes in agreement with [23] who found that fungating mass was the main colonoscopic picture in 86.2 % of cases. In the present study, as regards the tumors site, rectal carcinoma (47.8 %) was nearly equal to colonic (52.2 %). This agrees with [17,30] who told that about half of their studied cases were rectal lesions. On the other hand, [13] reported a lower incidence of rectal lesions (25.5 - 30.5 %) compared to colonic, while **Khafagy and his colleagues, (2000)** reported that rectal carcinoma was more common (68 %) than colonic ones.

Upon histopathological examination, most of the studied cases in the present study were adenocarcinoma (76.1 %), mucinous and signet ring adenocarcinomas constituted only 21.7 %. This comes in agreement with [31] who found that histologic tumor type was predominantly adenocarcinoma (78.5 %) while the more aggressive mucinous and signet ring adenocarcinomas constituted only (21.5 %)of their studied cases. This was also in agreement with [8,10,15,32]. In the current study, The fecal levels of tumor M2-PK were significantly higher ($P < 0.001$) in the studied cases with CRC (35.66 U/ml) than in the control group (2.74 U/ml). This was in agreement with, [14,33,34,35]. The median fecal tumor M2-PK level in the control group was 0.7 U/ml (range 0.02 - 17.4 U/ml) and in the studied cases was 36 U/ml (range 0.3 - 81U/ml). These findings come close to what was found by[36,14,34] who found that the median fecal tumor M2-PK level in the control group was 0.8, 2.10 and 1.75 U/ml, respectively; and the median level in CRC patients was 14.7, 22 and 11.72 U/ml, respectively.

In the present study, at a cut-off value of **3.9** U/ml, fecal tumor M2-PK was 97.5 % sensitive and 85 % specific for diagnosis of CRC with an AUROC = 0.96. [37] reported that M2PK had a sensitivity ranging from (73 - 97 %) and specificity from (78.6 - 100 %). [14] told that the high sensitivity of the tumor M2-PK test is due to its ability to detect bleeding and non-bleeding tumors. From a practical point of view, the use of a single random formed stool sample for tumor M2-PK analysis, without requiring dietary restrictions, might be of greater patient convenience.[14] The present study found that **fecal tumor M2PK ≥ 36.5 U/ml** could significantly discriminate cases with grade III adenocarcinoma with 90 % sensitivity and 63.9 % specificity, with AUROC = 0.76, while at **level ≥ 25.7 U/ml** it could significantly discriminate cases with Duke stage > A with 83.7 % sensitivity and 100 % specificity, with AUROC = 0.89. At **level ≥ 35.5 U/ml**it could significantly discriminate cases > T2 with 68% sensitivity and 61.9% specificity, with AUROC = 0.71, and at **level ≥ 50 U/ml**, it could significantly discriminate cases > N1 with 75 % sensitivity and 89.2 % specificity, with AUROC = 0.83.this is in agreement with [14,34]

Fecal tumor M2-PK level showed a statistically significant positive correlation with the pathological grades. The mean value of grade I was (6.25 U/ml), grade II (33.79 U/ml) and grade III (47.9 U/ml) ($P < 0.05$). This was in agreement with [14,33]

In the present study, the mean value of fecal tumor M2-PK level showed a statistically significant difference between different TNM stages ($P < 0.05$) with a statistically significant positive correlation. The mean values in T1, T2, T3 and T4 were 11.80, 31.87, 40.23 and 53 U/ml, respectively. This comes in agreement with [33] who found the mean value of T1, T2, T3 and T4 were 11.1, 23.8, 55.5 and 132.7 U/ml, respectively.

The mean value of fecal tumor M2-PK level showed a statistically significant difference between different Duke's stages ($P < 0.05$) in the present study with a statistically significant positive correlation. The mean values in Duke's A, B and C were 12.7, 33.33 and 42.23 U/ml, respectively. This was similar to [33] who found the mean value of Duke's A, B and C were 19.4, 31.5 and 57.1 U/ml, respectively

In the current study, there was a statistically significant correlation between Tumor M2-PK level with BMI and diabetes mellitus. M2-PK levels were significantly lower in diabetic patients than non-diabetics but no significant correlation with smoking. This was in agreement with [38]. In the current study, no statistically significant correlation was found between Tumor M2-PK level and either age or sex. This comes in agreement with [32] who assessed 32 CRC patients with a median age 66 Ys (male to female ratio was 3:1) and found no association between fecal tumor M2-PK level and patients' age or sex.

In the present study, there was a statistically significant negative correlation between tumor M2-PK level and serum albumin in the studied cases (r = - 0.33 and $P < 0.05$).This is in agreement with [34]. In the present study, there was a statistically highly significant positive correlation between tumor M2-PK level and both serum CEA and CA19-9 levels. However, fecal M2-PK was more sensitive than CEA and CA19.9 in detection of CRC. This comes in agree with [39,40] who said When other biomarkers such as CA19-9, CEA and M2-PK were compared, the sensitivity of M2-PK was 70%. But M2-PK showed the best results in CRC with a higher sensitivity when compared with CEA and CA19-9. They also concluded that M2-PK should be used in combination with CEA to increase the sensitivity. Also this is in agreement with [40,41,42,43] who told that the pooled sensitivity of CEA for diagnosis of CRC was only 46 % and the specificity was 89 %.

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