Evaluation of Plasma M2-Pyruvate Kinase as A Novel Biomarker in The Differentiation Between Functional and Organic Colonic Disorders

Original Article

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ABSTRACT

Background: It is challenging to distinguish between functional and organic colonic disorders because they can exhibit similar symptoms, with the exception of organic disorders that exhibit red flag symptoms including anemia, weight loss or bleeding per rectum. Therefore, a number of experiments were conducted to identify non-invasive methods for distinguishing between organic and functional colonic diseases.

Aim of the Work: Show the ability of plasma M2-pyruvate kinase as a new bio-marker for distinguishing between functional and organic bowel diseases and to evaluate its effectiveness in the latter.

Patients and Methods: Eighty participants were divided up into four groups: Group I: twenty individuals with functional disoders, which served as a control group. Group II: Twenty patients with inflammatory bowel diseases. Group III: Twenty patients with colonic polyps. Group IV: 20 colon cancer patients.

Results: The findings demonstrated that, at a cut-off level > 3 U/ml, serum M2-PK exhibited 100% specificity and 93.3% sensitivity in distinguishing between functional and organic colonic diseases. Additionally, at a cut-off level of > 12 U/ml, it demonstrated 100% sensitivity and 100% specificity in differentiating between benign (colonic polyp) and malignant lesions (CRC). Serum M2 PK and CEA showed highly substantial positive associations, and their combination showed improved sensitivity and specificity.

Conclusion: Serum M2-PK is able to distinguish between organic and functional bowel diseases. In order to minimize unnecessary endoscopic procedures, it can also be regarded as a non-invasive marker for screening for various organic colonic diseases .

Key Words: Colorectal Cancer, colorectal polyps, serum M2-Pyruvate Kinase.

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INTRODUCTION

Pyruvate Kinase enzymes play a role in glucose metabolism by converting phosphoenol-pyruvate to pyruvate and present in an organ-specific iso-enzymes (L, M1, M2 and R). In the normal cells, M2-Pyruvate Kinase (M2-PK) is tetra-meric isomer, highly avid for phosphoenol-pyruvate while the M2-PK iso-enzyme is dimeric, found in neoplastic cells, not avid for phosphoenol-pyruvate. Direct interaction between M2-PK and several onco-protein receptors causes the tetrameric to dimeric form of tumor cells to dissociate. Thus, tumor M2-PK is the name given to the dimeric M2-PK which can be released from tumor cells (due to its low avidity for phosphoenol-pyruvate) so, it can be detected in the body fluids[1].

Because M2-PK is a co-activator of transcription factors, it plays a significant role in the pathophysiology of tumors[2]. Therefore, by controlling glycolysis, energy production, and synthetic processes, it is a crucial enzyme for tumor growth[3].

Since tumor cells produce a lot of M2-PK and release it directly into various bodily fluids, it can be found in bodily fluids including blood and feces. The general public is more likely to comply with blood testing since they are more convenient than stool tests[4].

Compared to the general population, M2-PK was four times higher in CRC patients[4]. M2-PK can distinguish

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between malignant and benign tumors of the colon which can reflect survival rate of patients[5].

Colonic tumors are common health problem in the world and Egypt. CRC is one of the common malignancies among Egyptians[6].

The majority of CRC cases can be prevented by early surveillance and removal of pre-neoplastic lesions[7]. The tumor's stage at diagnosis typically has a significant impact on survival[8].

Despite the fact that colonoscopy is the most reliable way to diagnose colorectal cancer, but it is costy and invasive[5]. so, it is important to find cheap and non invasive tests with high sensitivity and specificity for detecting CRC[8].

It could be challenging to distinguish between functional colon illnesses like IBS and organic colon disorders like IBD because they can have similar symptoms. The diagnosis of IBD usually requires invasive measures as colonoscopy to visualize the mucosa and confirm diagnosis by histology. However, this can miss other diseases not directly visualized by colonoscopy. Also, IBD disease activity usually need repeated endoscopy as symptoms can correlate poorly with different stages of IBD activity[9].So, non-invasive serum bio-markers in IBD are very important for the diagnosis and assessing disease activity. They also can differentiate between organic and functional colon disorders by examining the entire GIT[10].

AIM OF THE WORK

This study aims to evaluate the diagnostic role of plasma M2-PK in distinguishing between organic (e.g., IBD, colo-rectal polyps, and cancer colon) and functional (e.g., IBS) colonic diseases, as well as its effectiveness in various organic colonic disorders.

PATIENTS AND METHODS

Between November 2017 and November 2019, 80 patients who met the study's inclusion requirements were treated at the Gastroenterology department's inpatient units and outpatient clinics at Ain Shams University and El Galaa Military Hospital.

Selection of patients:

The inclusion criteria: (1) subjects ≥18 years. (2) subjects with lower GI symptoms with indication for colonoscopy as chronic diarrhea or constipation, bleeding, unexplained weight loss and anemia. (3) the requirements for IBS: diagnosis confirmed by Rome IV criteria after all diagnostic work up (negative colonoscopy with histopathology). (4) The requirements for the IBD group: diagnosis was verified by clinical, colonoscopic, radiological and histo-pathological criteria. (5) The requirements for Colo-rectal polyp: diagnosed by colonoscopy and biopsy (6) The requirements for cancer colon group: diagnosis by colonoscopy and biopsy.

Subjects with dyspepsia or GERD, past or present history of chemotherapy or any treatment for colo-rectal cancer, patients with extra-colonic malignancy or Sepsis and subjects who refused to share in our study were excluded from this study.

Four groups of 80 patients were formed:

Group I: 20 subjects with functional colonic disorders (IBS group as a control group) as evidenced by negative colonoscope.

Group II: 20 patients with IBD with 18 patients with ulcerative colitis and 2 patient with Crohn’s disease.

Group III: 20 patients with colonic polyps (17 with adenomatous polyp and 3 with non-adenomatous polyp).

Group IV: 20 patients with CRC.

ETHICAL APPROVAL

The study's purposes were explained to all patients, and each participant provided written consent. The Ain Shams University Ethical Committee gave their approval to the study (FMASU MD 435/2017) at date 7/12/2017.

Every participant underwent the following tests: Laboratory tests such as stool analysis, fecal occult blood test (FOBT), complete blood count (CBC), erythrocyte

sedimentation rate, and C-reactive protein, as well as liver and kidney function tests and tumor markers like carcino-embryonic antigen (CEA) and carbohydrate antigen (CA 19-9) were used. The plasma M2-PK level was assessed using ELISA using two monoclonal antibodies that react with M-2PK without reacting with the other iso-forms of pyruvate kinase (L, R, M1, and M2 types).

All patients were subjected to Imaging studies as Abdomino-pelvis Ultrasound and CT pelvi-abdomen with contrast and Colonoscopy was biopsies taken for histopathological examination.

Statistical Analysis

The Statistical Package for Social Science (IBM SPSS) version 23 was used to gather and analyze the data. The quantitative data were presented as median with inter-quartile range (IQR) for non-parametric distributions and mean, standard deviations, and ranges for parametric distributions. Qualitative values were displayed as percentages and numbers.

The Chi-square test was used to compare the four groups. The Independent t-test was used to compare two independent groups with quantitative data. The Mann-Whitney test was used for data having a non-parametric distribution. When comparing more than two groups, the One Way ANOVA test was used. When dealing with non-parametric data, the Kruskall-Wallis test was used. The correlation between two quantitative factors in the same group was assessed using Spearman correlation coefficients. The ROC curve's sensitivity, specificity, PPV, NPV, and area under the curve (AUC) were used to establish the optimal cutoff level.

Table 1: Comparison between the Studied Groups as Regard age and sex.

		IBS group	IBD group	Colorectal polyps	CRC	Test value*	*P-value*	Sig.
		No. = 20	No. = 20	No. = 20	No. = 20			
Age (years)	Mean ± SD	40.5 ± 11.1	33.5 ± 10.83	44.95 ± 12.62	50.55 ± 10.49	8.149•	**0.000**	**HS**
	Range	20–57	18–55	18–65	23 – 69			
Sex	Male	11 (55.0%)	8 (40.0%)	13 (65.0%)	11 (55.0%)	2.564*	0.464	NS
	Female	9 (45.0%)	12 (60.0%)	7 (35.0%)	9 (45.0%)			
		Post hoc analysis						
	P1	P2	P3	P4	P5	P6		
Age (years)	0.054	0.216	0.006	0.002	0.000	0.121		

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
*: Chi-square test; •: One Way ANOVA test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

This table shows :-

- There was high statistical significant difference between studied groups as regard age .
- There was high statistical significant difference between (IBS vs CRC) & (IBD vs colorectal polyps) & (IBD vs CRC) & (colorectal polyps vs CRC) regarding age with No statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) regarding age.
- There is no statistical significant difference between studied groups as regard sex .

Table 2: Comparison between the Studied Groups as Regard main clinical presentation.

		IBS group	IBD group	Colorectal polyps	CRC	Test value*	*P-value*	Sig.
		No. = 20	No. = 20	No. = 20	No. = 20			
Abdominal pain	Negative	5 (25.0%)	13 (65.0%)	15 (75.0%)	17 (85.0%)	17.707*	**0.001**	**HS**
	Positive	15 (75.0%)	7 (35.0%)	5 (25.0%)	3 (15.0%)			
Bleeding per rectum	Negative	20 (100.0%)	13 (65.0%)	11 (55.0%)	14 (70.0%)	11.285*	**0.010**	**S**
	Positive	0 (0.0%)	7 (35.0%)	9 (45.0%)	6 (30.0%)			
Constipation	Negative	11 (55.0%)	19 (95.0%)	19 (95.0%)	20 (100.0%)	22.240*	**0.000**	**HS**
	Positive	9 (45.0%)	1 (5.0%)	1 (5.0%)	0 (0.0%)			
Diarrhea	Negative	13 (65.0%)	10 (50.0%)	20 (100.0%)	20 (100.0%)	22.932*	**0.000**	**HS**
	Positive	7 (35.0%)	10 (50.0%)	0 (0.0%)	0 (0.0%)			
Weight loss	Negative	20 (100.0%)	18 (90.0%)	17 (85.0%)	9 (45.0%)	21.875*	**0.000**	**HS**
	Positive	0 (0.0%)	2 (10.0%)	3 (15.0%)	11 (55.0%)			
Change of bowel habits	Negative	16 (80.0%)	19 (95.0%)	16 (80.0%)	16 (80.0%)	2.480*	0.479	NS
	Positive	4 (20.0%)	1 (5.0%)	4 (20.0%)	4 (20.0%)			
Pallor	Negative	20 (100.0%)	7 (35.0%)	6 (30.0%)	6 (30.0%)	28.168*	**0.000**	**HS**
	Positive	0 (0.0%)	13 (65.0%)	14 (70.0%)	14 (70.0%)			
		Post hoc analysis						
	P1	P2	P3	P4	P5	P6		
Abdominal pain	0.011	0.002	0.000	0.490	0.144	0.429		
Bleeding per rectum	0.004	0.001	0.008	0.518	0.736	0.327		
Constipation	0.003	0.003	0.001	1.000	0.311	0.311		
Diarrhea	0.337	0.004	0.004	0.000	0.000	–		
Weight loss	0.147	0.072	0.000	0.632	0.002	0.008		
Pallor	0.000	0.000	0.000	0.736	0.736	1.000		

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
*: Chi-square test; •: One Way ANOVA test; ≠: Kruskal-Wallis test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

This table shows:-
- High statistical significant difference between studied groups as regard abdominal pain, constipation,diarrhea, weight loss and pallor.
- statistical significant difference between studied groups as regard bleeding per rectum.
- No statistical significant difference between studied groups as regard change of bowel habits.

Table 3: Comparison between the Studied Groups as Regard ESR and CRP.

		IBS group	IBD group	Colorectal polyps	CRC	Test value*	*P-value*	Sig.
		No. = 20	No. = 20	No. = 20	No. = 20			
ESR (mm)	Median (IQR)	7 (3.5 – 9.5)	15.5 (11 – 24)	16 (12.5 – 18.5)	31 (17 – 48)	40.510≠	**0.000**	**HS**
	Range	2 – 17	5 – 36	4 – 34	11 – 70			
CRP (mg/l)	Median (IQR)	4 (3 – 5)	13.5 (8 – 22.5)	5 (3.5 – 13)	14.5 (11 – 28)	32.962≠	**0.000**	**HS**
	Range	2 – 7	4 – 48	2 – 35	3 – 45			
				Post hoc analysis				
	P1	P2	P3	P4	P5		P6	
ESR (mm)	0.000	0.000	0.000	0.957	0.003		0.002	
CRP (mg/l)	0.000	0.072	0.000	0.005	0.533		0.003	

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
*: Chi-square test; •: One Way ANOVA test; ≠: Kruskal-Wallis test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

This table shows:-

- There was high statistical significant difference between studied groups as regard ESR and CRP.
- There was high statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBS vs CRC) &(IBD vs CRC) &(colorectal polyps vs CRC) regarding ESR with no statistical significant difference between (IBD and colorectal polyps) regarding ESR.
- There was high statistical significant difference between (IBS vs IBD) & (IBS vs CRC) & (IBD and colorectal polyps) (colorectal polyps vs CRC) regarding CRP with no statistical significant difference between (IBS vs colorectal polyps) &(IBD vs CRC) regarding CRP.

Table 4: Comparison between the Studied Groups as Regard laboratory data (CBC, liver functions and renal functions).

		IBS group	IBD group	Colorectal polyps	CRC	Test value•	*P-value*	Sig.
		No. = 20	No. = 20	No. = 20	No. = 20			
Hb (g/dl)	Mean ± SD	12.28 ± 1.51	9.66 ± 1.31	10 ± 1.43	9.83 ± 1.14	16.505	**0.000**	**HS**
	Range	10.3 – 16	7.7 – 11.8	7.2 – 13	7.4 – 11.7			
PLT (thousand/ cmm)	Mean ± SD	273.65 ± 76.93	354.7 ± 114.72	268.9 ± 84.06	287.6 ± 98.75	3.529	**0.019**	**S**
	Range	178 – 444	173 – 567	156 – 451	23 – 456			
WBCs (thousand/ cmm)	Mean ± SD	6.48 ± 2.13	6.71 ± 2.05	5.94 ± 1.44	6.42 ± 1.32	0.668	0.574	NS
	Range	4.2 – 11	4.5 – 11.1	4.5 – 10	4.7 – 9.6			
ALT (IU/L)	Mean ± SD	27.9 ± 7.25	27.55 ± 8.51	30.65 ± 6.98	30.75 ± 5.25	1.182	0.322	NS
	Range	10 – 43	11 – 43	21 – 45	22 – 39			
AST (IU/L)	Mean ± SD	27.75 ± 6.32	30.4 ± 5.43	26.05 ± 6.79	31 ± 8.69	2.244	0.090	NS
	Range	17 – 38	23 – 44	10 – 39	11 – 42			
Albumin (g/dl)	Mean ± SD	3.91 ± 0.4	3.83 ± 0.55	3.89 ± 0.4	3.88 ± 0.66	0.101	0.959	NS
	Range	3.1 – 4.5	2.7 – 5	3.4 – 4.6	3 – 5.6			
INR	Mean ± SD	1.07 ± 0.07	1.07 ± 0.07	1.06 ± 0.06	1.07 ± 0.08	0.044	0.988	NS
	Range	0.96 – 1.2	0.96 – 1.2	0.98 – 1.2	0.96 – 1.2			
Bilirubin T (mg/dl)	Mean ± SD	0.73 ± 0.29	0.68 ± 0.25	0.66 ± 0.28	0.69 ± 0.28	0.271	0.846	NS
	Range	0.3 – 1.2	0.3 – 1.1	0.3 – 1.2	0.3 – 1.2			
Urea (mg/dl)	Mean ± SD	23.6 ± 8.63	25.25 ± 5.35	28.85 ± 6.83	24.4 ± 6.59	2.223	0.092	NS
	Range	0 – 34	11 – 35	12 – 40	12 – 34			
S.creatinine (mg/ dl)	Mean ± SD	0.76 ± 0.28	0.92 ± 0.41	0.74 ± 0.39	0.62 ± 0.47	2.039	0.116	NS
	Range	0.3 – 1.3	0.2 – 1.66	0.2 – 1.5	0.2 – 1.5			
		Post hoc analysis						
	P1	P2	P3	P4	P5	P6		
Hb (g/dl)	0.000	0.000	0.000	0.423	0.684	0.693		
PLT (thousand/ cmm)	0.008	0.874	0.643	0.005	0.028	0.534		

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
•: One Way ANOVA test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

This table shows:-
- High statistically significant difference between studied groups as regard Hb .
- statistical significant difference between studied groups as regard platelets .
- No statistical significant difference between studied groups as regard WBCs, ALT, AST, albumin, INR, bilirubin, urea and S.creatinine.

Table 5: Comparison between the Studied Groups as Regard CEA, CA19-9 and plasma M2 PK.

		IBS group	IBD group	Colorectal polyps	CRC	Test value*	*P-value*	Sig.
		No. = 20	No. = 20	No. = 20	No. = 20			
CEA (ng/mL)	Median (IQR)	0.85(0.7– 1)	5.3(3.4 – 7)	4.8(3.84 – 9.1)	79.05(18.5 – 200)	57.974≠	**0.000**	**HS**
	Range	0.2 – 4.3	1 – 9	1.9 – 15	5 – 893			
CA19-9 (IU/mL)	Mean ± SD	23.30± 10.68	25.95± 11.67	29.10 ± 17.24	33.95 ± 26.45	1.345•	0.266	NS
	Range	9.00 – 47.00	9.50 – 55.00	5.50 – 65.00	7.00 – 115.00			
Plasma M2 PK (IU/mL)	Mean ± SD	1.34 ± 0.71	5.18 ± 2.74	8.05 ± 2.55	18.93 ± 5.87	93.254•	**0.000**	**HS**
	Range	0.5 – 3	1.9 – 13.5	3.9 – 12	12.4 – 29			
				Post hoc analysis				
	P1	P2	P3	P4	P5		P6	
CEA (ng/mL)	0.000	0.000	0.000	0.645	0.000		0.000	
Plasma M2 PK (IU/mL)	0.001	0.000	0.000	0.011	0.000		0.000	

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
•: One Way ANOVA test; ≠: Kruskal-Wallis test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

This table shows:-
- High statistical significant difference between studied groups as regard CEA and Plasma M2 PK.
- No statistical significant difference between studied groups as regard CA19-9.
- High statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBS vs CRC) &(IBD vs CRC) &(colorectal polyps vs CRC) regarding CEA and No statistical significant difference between (IBD and colorectal polyps) regarding CEA.
- High statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBS vs CRC) &(IBD vs CRC) &(colorectal polyps vs CRC) regarding Plasma M2 PK and statistical significant difference between (IBD and colorectal polyps) regarding Plasma M2 PK.

Table 6: Comparison between the Studied Groups as Regard stool analysis and FOBT.

		IBS group	IBD group	Colorectal polyps	CRC	Test value*	*P-value*	Sig.
		No. = 20	No. = 20	No. = 20	No. = 20			
Stool analysis	Normal	11(55.0%)	2(10.0%)	3(15.0%)	6(30.0%)	25.842•	**0.011**	**S**
	Amebic cyst	5(25.0%)	1(5.0%)	4(20.0%)	2(10.0%)			
	Undigested food	3(15.0%)	2(10.0%)	3(15.0%)	4(20.0%)			
	WBCs	1(5.0%)	5(25.0%)	2(10.0%)	2(10.0%)			
	RBCs	0(0.0%)	10(50.0%)	8(40.0%)	6(30.0%)			
FOBT	Negative	20 (100.0%)	8 (40.0%)	12 (60.0%)	9 (45.0%)	18.697*	**0.000**	**HS**
	Positive	0 (0.0%)	12 (60.0%)	8 (40.0%)	11 (55.0%)			
				Post hoc analysis				
	P1	P2	P3	P4	P5	P6		
FOBT	0.000	0.002	0.000	0.206	0.749	0.342		
Stool analysis	0.000	0.011	0.055	0.446	0.259	0.718		

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
*: Chi-square test; •: One Way ANOVA test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

- This table shows:-
- High statistical significant difference between studied groups as regard FOBT .
- Statistical significant difference between studied groups as regard stool analysis especialy regarding WBCs and RBCs in stool .
- High statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBS vs CRC) regarding FOBT as (*P-value* < 0.01) and no statistical significant difference between (IBD and colorectal polyps) & (IBD vs CRC) &(colorectal polyps vs CRC) regarding FOBT .
- High statistical significant difference between (IBS vs IBD) regarding stool analysis as (*P-value* < 0.01) and statistical significant difference between (IBS vs colorectal polyps) regarding stool analysis and no statistical significant difference between (IBS vs CRC) & (IBD and colorectal polyps) & (IBD vs CRC) & (colorectal polyps vs CRC) regarding stool analysis .

Table 7: Comparison between the Studied Groups as Regard imaging.

			IBS group	IBD group	Colorectal polyps	CRC	Test value*	*P-value*	Sig.
			No. (%)	No. (%)	No. (%)	No. (%)			
Abdomen and pelvis u/s	*Normal		14 (70.0%)	14 (70.0%)	17 (85.0%)	8 (40.0%)			
	*Hepatomegaly &colonic gases distension		6 (30.0%)	6 (30.0%)	3 (15.0%)	3 (15.0%)			
			0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (15.0%)			
	*Masses	LNS mass	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (10.0%)	32.226	**0.006**	**HS**
		Ascites & HFL &LNs	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.0%)			
		RT sided abd mass	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (15.0%)			
		LT sided abd mass							
CT abdomen and pelvis	*Normal		14 (70.0%)	13 (65.0%)	13 (65.0%)	0 (0.0%)			
	*Hepatomegaly&colonic gases distension		6 (30.0%)	5 (25.0%)	3 (15.0%)	2 (10.0%)			
	*Colonic soft tissue lesion		0 (0.0%)	2 (10.0%)	4 (20.0%)	0 (0.0%)			
	*Masses	Rectal mass lesion	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.0%)			
		Mural thickening at tranverse colon.	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.0%)	77.233	**0.000**	**HS**
		Mural thickening at the LT side of the colon.	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (55.0%)			
		Mural thickening at the RT side of the colon.	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (20.0%)			
		Mass at the LT side of the colon.	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.0%)			

	Post hoc analysis					
	P1	P2	P3	P4	P5	P6
Abdomen and pelvis u/s	1.000	0.256	0.040	0.256	0.040	0.032
CT abdomen and pelvis	0.345	0.081	0.000	0.558	0.000	0.000

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
*: Chi-square test; •: One Way ANOVA test; ≠: Kruskal-Wallis test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

This table shows:-

- High statistical significant difference between studied groups as regard abdomen and pelvis u/s & CT abdomen and pelvis.
- statistical significant difference between (IBS vs CRC) & (IBD vs CRC) & (colorectal polyps vs CRC) regarding abdomen and pelvis u/s and No statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBD and colorectal polyps) regarding abdomen and pelvis u/s.
- High statistical significant difference between (IBS vs CRC) & (IBD vs CRC) &(colorectal polyps vs CRC) regarding CT abdomen and pelvis as (P-value < 0.01) and No statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBD and colorectal polyps) regarding CT abdomen and pelvis.

Table 8: Comparison between the Studied Groups as Regard colonoscopy, histopathology and grading.

			IBS group	IBD group	Colorectal polyps	CRC	Test value*	*P-value*	Sig.
			No. (%)	No. (%)	No. (%)	No. (%)			
	*IBS	Normal	20(100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
	*IBD (congestion & Ulceration)	Normal or inactive disease	0 (0.0%)	6 (30.0%)	0 (0.0%)	0 (0.0%)			
		Mild disease	0 (0.0%)	7 (35.0%)	0 (0.0%)	0 (0.0%)			
		Moderate disease	0 (0.0%)	4 (20.0%)	0 (0.0%)	0 (0.0%)			
		Severe disease	0 (0.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)			
		Skip lesions	0 (0.0%)	2 (10.0%)	0 (0.0%)	0 (0.0%)			
		Polyps at the rectum	0 (0.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)			
Colonoscopy	* polyps	Polyps at the distal colon	0 (0.0%)	0 (0.0%)	7 (35.0%)	0 (0.0%)	240.000	**0.000**	**HS**
		Polyps at the proximal colon	0 (0.0%)	0 (0.0%)	8 (40.0%)	0 (0.0%)			
		Polyps at the Proximal and the distal colon	0 (0.0%)	0 (0.0%)	3 (15.0%)	0 (0.0%)			
	* CRC (masses)	Mass at the rectum	0 (0.0%)	0 (0.0%)	0 (0.0%)	6(30.0%)			
		Mass at the distal colon	0 (0.0%)	0 (0.0%)	0 (0.0%)	8(40.0%)			
		Mass at the proximal colon	0 (0.0%)	0 (0.0%)	0 (0.0%)	6(30.0%)			
histopathology	*IBS	Normal	20(100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
	*IBD	Ulcerative colitis	0 (0.0%)	18(90.0%)	0 (0.0%)	0 (0.0%)			
		Colonic crohns disease	0 (0.0%)	2 (10.0%)	0 (0.0%)	0 (0.0%)			
	*polyps	Tubular adenoma	0 (0.0%)	0 (0.0%)	14(70.0%)	0 (0.0%)			
		Tubulovillous adenoma	0 (0.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	240.000	**0.000**	**HS**
		Villous adenoma	0 (0.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)			
		Hyperplastic polyp	0 (0.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)			
	*CRC	Adenocarcinoma	0 (0.0%)	0 (0.0%)	0 (0.0%)	17(85.0%)			
		Mucinous adenocarcinoma	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (15.0%)			
Grading	*IBS &IBD	No grading	20(100.0%)	20(100.0%)	0 (0.0%)	0 (0.0%)			
	*polyps	Low-risk adenomas	0 (0.0%)	0 (0.0%)	9 (45.0%)	0 (0.0%)			
		High-risk adenomas	0 (0.0%)	0 (0.0%)	9 (45.0%)	0 (0.0%)			
		Non adenomatous polyp	0 (0.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	160.000	**0.000**	**HS**
	*CRC	Well differentiated	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (10.0%)			
		Moderatly differentiated	0 (0.0%)	0 (0.0%)	0 (0.0%)	13(65.0%)			
		Poorly differentiated	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (25.0%)			
			Post hoc analysis						
	P1	P2	P3	P3	P4	P5	P6		
Colonoscopy	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
histopathology	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
Grading	–	0.000	0.000	0.000	0.000	0.000	0.000		

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
*: Chi-square test; •: One Way ANOVA test; ≠: Kruskal-Wallis test
P1: IBS vs IBD group P2: IBS vs colorectal polyps group
P3: IBS vs CRC group P4: IBD vs colorectal polyps group
P5: IBD vs CRC group P6: Colorectal polyps vs CRC group

This table shows:-

- High statistical significant difference between studied groups as regard colonoscopy, histopathology and grading.
- High statistical significant difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBS vs CRC) &(IBD and colorectal polyps) & (IBD vs CRC) & (colorectal polyps vs CRC) regarding colonoscopy, histopathology and grading.

Table 9: Correlations between Plasma M2 PK level and other parameters in all cases.

	Plasma M2 PK	
Variant	r	*P-value*
Age (years)	**0.312****	**0.005**
ESR (mm)	**0.652****	**0.000**
CRP (mg/l)	**0.402****	**0.000**
Hb (g/dl)	**-0.445****	**0.000**
PLT (thousand/cmm)	-0.070	0.539
WBCs (thousand/cmm)	0.070	0.535
ALT (IU/L)	0.157	0.164
AST (IU/L)	0.182	0.106
Albumin (g/dl)	-0.070	0.538
INR	-0.063	0.581
Bilirubin T (mg/dl)	-0.038	0.740
Urea (mg/dl)	-0.024	0.833
S.creatinine (mg/dl)	-0.215	0.056
CEA (ng/mL)	**0.787****	**0.000**
CA19-9 (IU/mL)	0.122	0.279

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
(r): Spearman correlation coefficient

This table showed the correlation between Plasma M2 PK level and other laboratory tests in all patients according to correlation coefficient (r) using Spearman's correlation coefficient test.

- There was a high significant positive correlations between Plasma M2 PK level and (age, ESR. CRP and CEA) in all patients.
- There was a high significant negative correlations between Plasma M2 PK level and Hb in all patients.

Table 10: Relations between Plasma M2 PK level and IBD group as regard colonoscopy finding.

variant		Plasma M2 PK		Test value	*p- value*	Sig.
		Mean ± SD	Range			
Colonoscopy	IBD group					
	• Ulcerative colitis					
	Normal or inactive disease (mayo score 0) (n=6)	3.38±1.41	1.9–5.6	15.898	0.000	**HS**
	Mild disease (mayo score 1) (n=7)	4.06±1.32	2.5–6.1			
	Moderate disease (mayo score 2) (n=4)	7.07±1.51	5.27–8.9			
	Severe disease (mayo score 3) (n=1)	13.5±0	13.5–13.5			
	• Colonic Crohns disease					
	Skip lesions (n=2)	6.55±0.07	6.5–6.6			

- This table shows that there was high statistical significant difference as regard relation of plasma M2–PK to endoscopic activity of ulcerative colitis as plasma M2-PK more elevated in mayo1 & 2 & 3 (active disease) in comparison to Mayo 0 (normal or in active disease) .

Table 11: Relations between Plasma M2 PK level and (IBD, colorectal polyps and CRC groups) as regard histopathological finding.

Variant		Plasma M2 PK		Test value	*p- value*	Sig
		Mean ± SD	Range			
Histopathology	**IBD group**					
	• Ulcerative colitis (n=18)	5.03±2.85	1.9–13.5	0.736	0.471	NS
	• Colonic crohns disease (n=2)	6.55±0.07	6.5–6.6			
	Colorectal polyps group					
	-Adenomatous polyp					
	• Tubular adenoma (n=14)	7.96±2.28	3.9–11.8	**4.077**	**0.025**	**S**
	• Tubulovillous adenoma (n=2)	8.95±1.34	8–9.9			
	• Villous adenoma (n=2)	11.5±0.71	11–12			
	-Non adenomatous polyp					
	• Hyperplastic polyp (n=2)	4.3±0.57	3.9–4.7			
	CRC group					
	• Adenocarcinoma (n=17)	17.3±4.69	12.4–26	**3.914**	**0.001**	**HS**
	• Mucinous adenocarcinoma (n=3)	28.17±1.04	27–29			

This table showed the following:-

- There was high statistical significant difference as regard relation of plasma M2-PK to CRC histopathological severity as it elevated in Mucinous adenocarcinoma more than non-Mucinous adenocarcinoma .
- There was statistical significant difference as regard relation of plasma M2 - PK to colorectal polyp histopathological severity as it elevated in adenomatous polyp (villous > tubulovillous > tubular) more than non-adenomatous polyp (hyperplastic polyp) .
- There was no statistical significant difference as regard relation of plasma M2-PK to IBD group as regard histopatholgy finding .

Table 12: Relations between Plasma M2 PK level and (colorectal polyps and CRC groups) as regard grading.

variant		Plasma M2 PK		Test value	*P- value*	Sig.
		Mean ± SD	Range			
Grading	**Colorectal polyps group**					
	Adenomatous polyp Low risk	6.79±1.63	3.9±9.5	**16.617**	**0.000**	**HS**
	High risk	10.14±1.59	8±12			
	Non adenomatous polyp	4.3±0.57	3.9±4.7			
	CRC group					
	Well differentiated	13.5±0.71	13±14	**13.457**	**0.000**	**HS**
	Moderatly differentiated	16.85±4.38	12.4±26			
	Poorly differentiated	26.5±2.4	24±29			

This table showed the following:-

- There was high statistical significant difference as regard relation of plasma M2- PK to colorectal polyp grading risk as it more elevated in adenomatous polyp (high risk adenoma > low risk adenoma) than non - adenomatous polyp (hyperplastic polyp).
- There was high statistical significant difference as regard relation of plasma M2-PK to CRC grading severity as it more elevated in (poorly differentiated > moderately differentiated > well differentiated colorectal cancer).

Table 13: Comparison between the functional and organic Groups as Regard (CEA,CA19-9, plasma M2 PK, stool analysis and FOBT).

		Functional group	Organic group	Test value	*P-value*	Sig.
		No. = 20	No. = 60			
CEA (ng/mL)	Median (IQR)	0.85 (0.7 – 1)	7 (4.27 – 19.5)	-6.258≠	**0.000**	**HS**
	Range	0.2 – 4.3	1 – 893			
CA19-9 (IU/mL)	Mean ± SD	23.30 ± 10.68	29.67 ± 19.39	-1.396•	0.167	NS
	Range	9.00 – 47.00	5.50 – 115.00			
Plasma M2 PK (IU/mL)	Mean ± SD	1.34 ± 0.71	10.72 ± 7.16	-5.826•	**0.000**	**HS**
	Range	0.5 – 3	1.9 – 29			
Stool analysis	Normal	11 (55.0%)	11 (18.3%)	18.311*	**0.001**	**HS**
	Amebic cyst	5 (25.0%)	7 (11.7%)			
	Undigested food	3 (15.0%)	9 (15.0%)			
	WBCs	1 (5.0%)	9 (15.0%)			
	RBCs	0 (0.0%)	24 (40.0%)			
FOBT	Negative	20 (100.0%)	29 (48.3%)	16.871*	**0.000**	**HS**
	Positive	0 (0.0%)	31 (51.7%)			

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant
*: Chi-square test; •: Independent t-test; ≠: Mann-Whitney test

This table showed :-

- There was High statistical significant difference between the functional and organic groups as regard CEA, Plasma M2 PK, stool analysis and FOBT .
- There was no statistical significant difference between the functional and organic groups as regard CA19-9 .

Table 14: Diagnostic performance of CEA, Plasma M2-PK and FOBT in Discrimination of functional and organic Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
CEA (ng/ml)	>1	0.969	98.33	85.00	95.2	94.4
Plasma M2 PK (IU/ml)	>3	0.992	93.33	100.00	100.0	83.3
FOBT	–	0.638	51.7	100.0	100.0	69.0

ROC curve: receiver operating characteristic curve
AUC: area under the curve
PPV: positive predictive value
NPV: negative predictive value

This table showed Diagnostic performance of CEA, Plasma M2-PK and FOBT as a marker in Discrimination between functional and organic groups using Receiver Operator characteristics Curve (ROC) test :

- Validity of CEA as a biomarker for the discrimination between functional and organic groups was shown in table (13): the cut off value of CEA (> 1 ng /ml) had sensitivity 98.33%, specificity 85%, PPV 95.2% and NPV 94.4% .
- Validity of Plasma M2-PK as a biomarker for discrimination between functional and organic groups was shown in table (13): the cut off value of Plasma M2-PK (> 3 IU /ml) had sensitivity 93.33%, specificity 100%, PPV 100% and NPV 83.3% .
- Validity of FOBT as a biomarker for discrimination between functional and organic groups was shown in table (13): sensitivity 51.7%, specificity 100%, PPV 100% and NPV 69% .

Table 15: Diagnostic performance of CEA and Plasma M2-PK in Discrimination between IBS and IBD Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
CEA (ng/ml)	>1	0.950	95.00	85.00	86.4	94.4
Plasma M2 PK (IU/ml)	>2	0.976	90.00	90.00	90.0	90.0

ROC curve: receiver operating characteristic curve AUC: area under the curve
PPV: positive predictive value NPV: negative predictive value

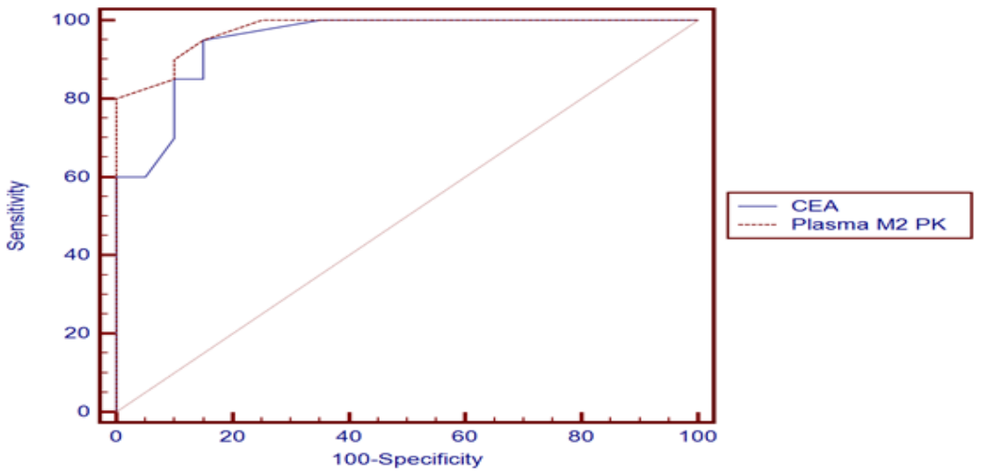

Fig. 1: Roc curve of CEA and Plasma M2 PK between IBS and IBD group in predicting of IBD group .

- The cut off value of CEA (> 1 ng /ml) had sensitivity 95%, specificity 85%, PPV 86.4% and NPV 94.4%.
- The cut off value of Plasma M2-PK (> 2 IU /ml) had sensitivity 90%, specificity 90%, PPV 90% and NPV 90%.

Table 16: Diagnostic performance of CEA and Plasma M2-PK in Discrimination between IBS and colorectal polyps Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
CEA (ng/ml)	>1	0.958	100.00	85.00	87.0	100.0
Plasma M2 PK (IU/ml)	>3	1.000	100.00	100.00	100.00	100.00

ROC curve: receiver operating characteristic curve AUC: area under the curve
PPV: positive predictive value NPV: negative predictive value

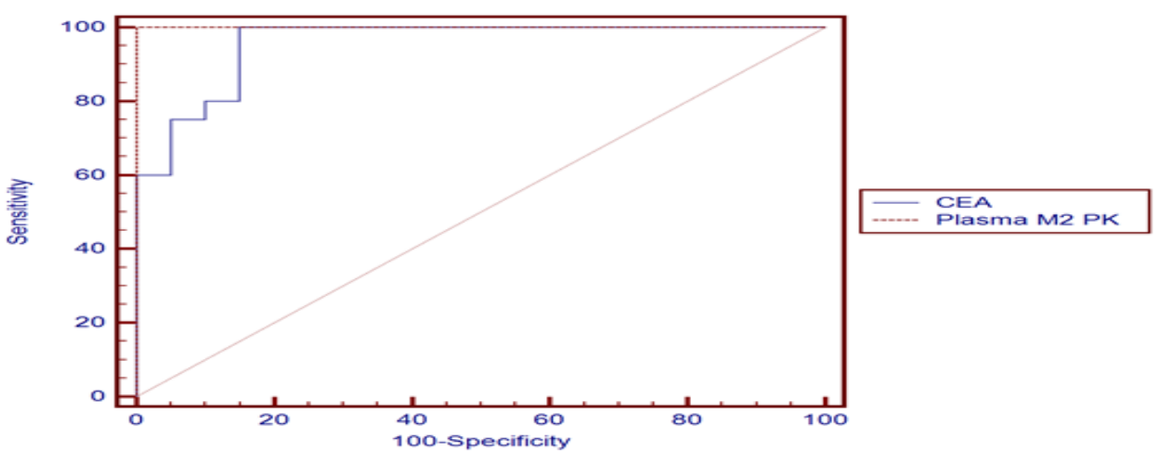

Fig. 2: Roc curve of CEA and Plasma M2 PK between IBS and Colorectal polyps group in predicting of Colorectal polyps group .

- The cut off value of CEA (> 1 ng /ml) had sensitivity 100%, specificity 85%, PPV 87% and NPV 100% .
- The cut off value of Plasma M2-PK (> 3 IU /ml) had sensitivity 100%, specificity 100%, PPV 100% and NPV 100% .

Table 17: Diagnostic performance of CEA and Plasma M2-PK in Discrimination between IBS and CRC Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
CEA (ng/ml)	>4.3	1.000	100.00	100.00	100.00	100.00
Plasma M2 PK (IU/ml)	>3	1.000	100.00	100.00	100.00	100.00

ROC curve: receiver operating characteristic curve AUC: area under the curve
PPV: positive predictive value NPV: negative predictive value

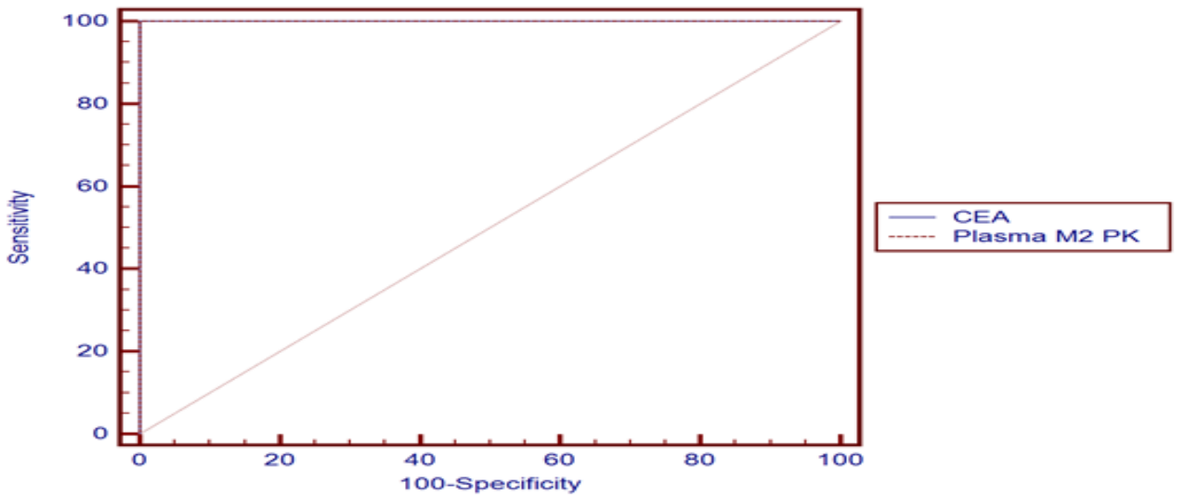

Fig. 3: Roc curve of CEA and Plasma M2 PK between IBS and CRC group in predicting of CRC group .

- The cut off value of CEA (> 4.3 ng /ml) had sensitivity 100%, specificity 100%, PPV 100% and NPV 100%.
- The cut off value of Plasma M2-PK (> 3 IU /ml) had sensitivity 100%, specificity 100%, PPV 100% and NPV 100%.

Table 18: Diagnostic performance of Plasma M2-PK in Discrimination between IBD and colorectal polyps Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
Plasma M2 PK (IU/ml)	>5.6	0.794	85.00	65.00	70.8	81.2

ROC curve: receiver operating characteristic curve AUC: area under the curve
PPV: positive predictive value NPV: negative predictive value

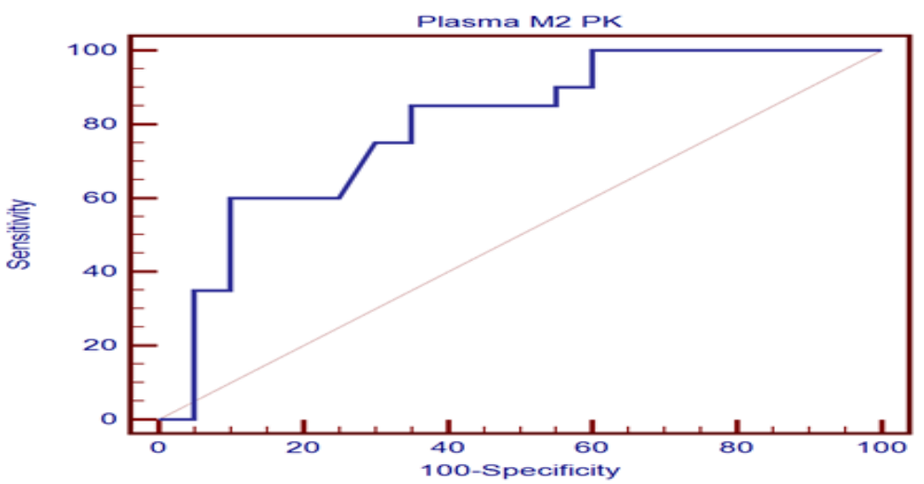

Fig. 4: Roc curve of Plasma M2 PK between IBD and Colorectal polyps group in predicting of Colorectal polyps group .

- The cut off value of Plasma M2-PK (> 5.6 IU /ml) had sensitivity 85%, specificity 65%, PPV 70.8% and NPV 81.2%.

Table 19: Diagnostic performance of CEA and Plasma M2-PK in Discrimination of IBD and CRC Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
CEA(ng/ml)	>9	0.956	85.00	100.00	100.0	87.0
Plasma M2 PK (IU/ml)	>8.9	0.991	100.00	95.00	95.2	100.0

ROC curve: receiver operating characteristic curve AUC: area under the curve
PPV: positive predictive value NPV: negative predictive value

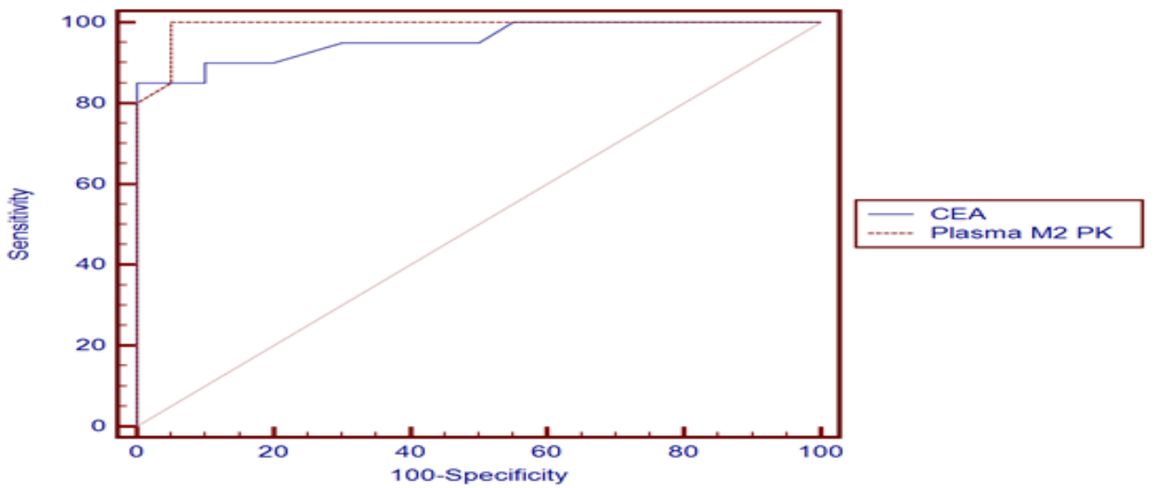

Fig. 5: Roc curve of CEA and Plasma M2 PK between IBD and CRC groups in predicting of CRC group .

- The cut off value of CEA (> 9 ng /ml) had sensitivity 85%, specificity 100%, PPV 100% and NPV 87%.
- The cut off value of Plasma M2-PK (> 8.9 IU /ml) had sensitivity 100%, specificity 95%, PPV 95.2% and NPV 100%.

Table 20: Diagnostic performance of CEA and Plasma M2-PK in Discrimination of colorectal polyps and CRC Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
CEA (ng/ml)	>15	0.930	75.00	100.00	100.0	80.0
Plasma M2 PK (IU/ml)	>12	1.000	100.00	100.00	100.00	100.00

ROC curve: receiver operating characteristic curve AUC: area under the curve
PPV: positive predictive value NPV: negative predictive value

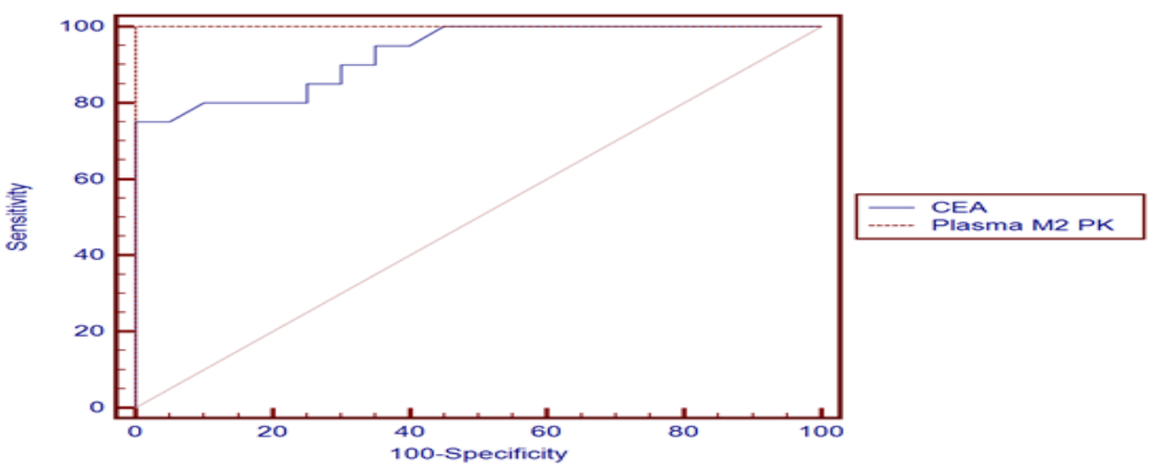

Fig. 6: Roc curve of CEA and Plasma M2 PK between Colorectal polyps and CRC groups in predicting of CRC group .

- The cut off value of CEA (> 15 ng /ml) had sensitivity 75%, specificity 100%, PPV 100% and NPV 80%.
- The cut off value of Plasma M2-PK (> 12 IU /ml) had sensitivity 100%, specificity 100%, PPV 100% and NPV 100%.

Table 21: Diagnostic performance of CEA, Plasma M2- PK and FOBT in Discrimination of functional and organic Groups.

Variable	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
CEA (ng/ml)	>1	0.969	98.33	85.00	95.2	94.4
Plasma M2 PK (IU/ml)	>3	0.992	93.33	100.00	100.0	83.3
FOBT	–	0.638	51.7	100.0	100.0	69.0

ROC curve: receiver operating characteristic curve AUC: area under the curve
PPV: positive predictive value NPV: negative predictive value

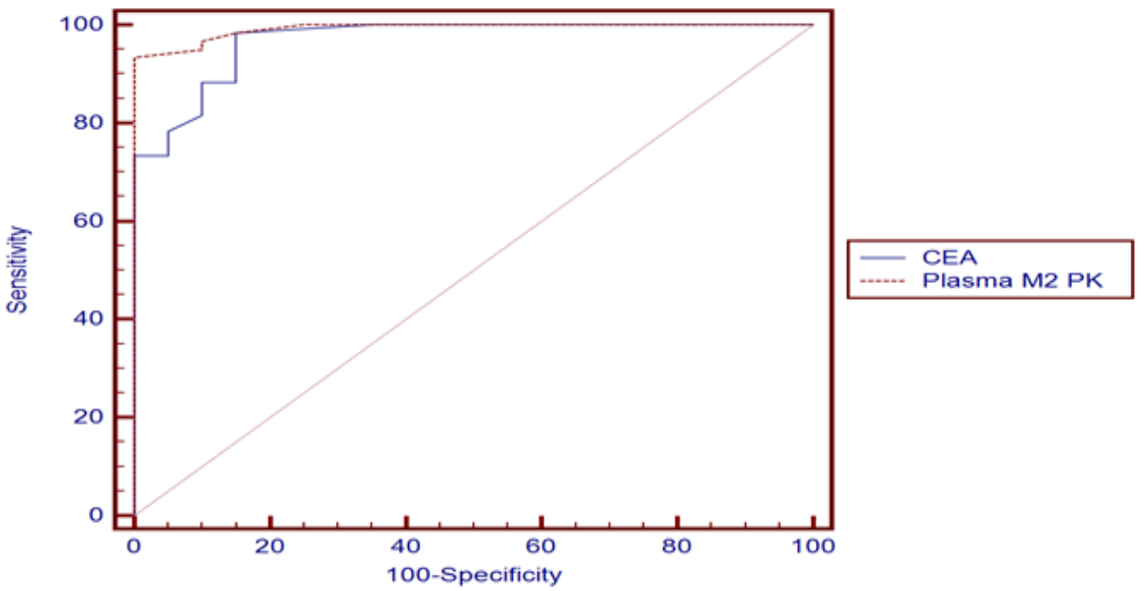

Fig. 7: Roc curve of CEA and Plasma M2 PK between functional and organic groups in predicting of organic group.

- The cut off value of CEA (> 1 ng /ml) had sensitivity 98.33%, specificity 85%, PPV 95.2% and NPV 94.4%.
- The cut off value of Plasma M2-PK (> 3 IU /ml) had sensitivity 93.33%, specificity 100%, PPV 100% and NPV 83.3%.
- Validity of FOBT as a marker of Discrimination between functional and organic groups had sensitivity 51.7%, specificity 100%, PPV 100% and NPV 69%.

DISCUSSION

Pyruvate kinases usually play a role in glucose metabolism by converting phosphoenol-pyruvate to pyruvate and usually present in an organ-specific iso-enzymes (L, R, M1, and M2 isoforms). M2PK isoenzyme is found in neoplastic cells with a low affinity for phosphoenol-pyruvate. So, the dimeric M2PK is called neoplastic M2PK which is easily released from neoplastic cells and can be quantitatively detected in the different body fluids as blood and stool. blood tests are more suitable than fecal tests with higher compliance among the general population.

Colonic neoplasms are common health problem in Egypt as CRC is one of the commonest malignancies in Egypt which can be prevented by early surveillance of colonic lesions. Although the gold standard diagnostic tool for detecting CRC is colonoscopy, but it is costy and invasive. So, it is so Important to find cheap and non invasive method to detect CRC or other precancerous lesions.

The thesis was done on 80 subjects who fulfilled inclusion criteria from the inpatient units and outpatient clinics of Gastroenterology department of Ain Shams University and El Galaa Military Hospital during the duration from November 2017 to November 2019. Those patients were divided into 4 groups:

Group I: 20 subjects with functional bowel disorders (IBS group as a control group) as evidenced by negative colonoscope.

Group II: 20 patients with IBD (18 with ulcerative colitis and 2 with Crohn's disease).

Group III: 20 patients with colonic polyps (17 with adenomatous polyp and 3 with non adenomatous polyp).

Group IV: 20 patients with Colo-rectal cancer.

Full history taking, Clinical examination, Laboratory investigations as Fecal occult blood test (FOBT), CBC, ESR and CRP, Liver function tests, Kidney function tests, CEA, CA 19-9 with Measurement of plasma M2-PK level by ELISA with imaging studies as Abdomino-pelvis ultrasound and CT pelvi-abdomen with contrast and Colonoscopy was biopsies taken for histopathological examination were done.

According to our research, there was a substantial statistical difference between the organic and functional groups in terms of CEA. In the functional group, the median CEA level was 0.85 ng/ml, but in the organic group, it was 7 ng/ml. Additionally, there was a significant statistical difference between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBS vs CRC) & (IBD vs CRC) & (colorectal polyps vs CRC) as regard CEA and no statistical significant difference between (IBD and colorectal polyps) regarding CEA. in our study, the cut off value of CEA (> 1 ng /ml) had sensitivity 98.33%, specificity 85%.

Our results in agreed with Fakih and Padmanabhan[11] and ***El-Gayar et al.***[12] and ***Elnadry et al.***[13]. CEA, the most commonly used tumour marker in CRC which had a low sensitivity for early-stage tumors[14]. ***Thomas et al.***[15] found that the level of CEA increased in a third of all cases who had late-stages of CRC, whereas level was static in both benign and non-cancer controls. Unfortunately, the available serum biomarkers for diagnosis and prognosis of CRC as CEA and CA19-9, are non sensitive[16]. AL-Janabi[17] concluded that patients with IBS had increased CEA level but within a limited range (<10 ng/mL) while The level higher more than 10 ng/ ml increases suspicion of CRC.

Kim et al.[18] found that CEA was increased in colorectal adenoma and CRC with correlation with the severity of colorectal neoplasm. Also, ***Polat et al.***[19] found that CEA increased with advancing stage of CRC with lymph node invasion and distant metastasis.

In our study, there was high statistical significance between the functional and organic groups as regard plasma M2-PK level. we found that average plasma M2-PK level ranged between (0.5 – 3 IU/mL) with Mean ± SD (1.34 ± 0.71 IU/mL) in the functional group and between (1.9 – 29 IU/mL) with Mean ± SD (10.72 ± 7.16 IU/mL) in the organic group. Also, there was high statistical significance between (IBS vs IBD) & (IBS vs colorectal polyps) & (IBS vs CRC) & (IBD vs CRC) & (IBD and colorectal polyps) & (colorectal polyps vs CRC) regarding Plasma M2 PK level. At a cut-off level > 3 U/ml, we discovered that plasma M2-PK has a 93.33% sensitivity and 100% specificity in distinguishing between functional and organic colonic diseases. This agreed with ***Wahib et al***.[20] which concluded that Plasma M2-PK can discrminate between functional and organic colonic disorders with 81.94% sensitivity, 83.3% specificity at a cut - off level > 3 (U/ml). This also agreed with with ***Bastawy et al.***[21] who revealed that level of M2-PK in the stool was increased markedly in patients with organic colonic disorders (IBD and CRC patients) than those functional group (IBS) with sensitivity (87.5%) and specificity (80%) at a cut-off value of 4.2 U/ml.

At a cut-off level of > 12 U/ml, this study demonstrated that plasma M2-PK may distinguish between benign (colo-rectal polyp) and malignant colonic cancers (CRC) with 100% sensitivity and 100% specificity which agreed with ***Wahib et al.***[20] and ***Meng et al.***[4] which found that Plasma M2-PK could discriminate between benign colonic polyps and CRC with 75.5% sensitivity, 87.5% specificity at a cut-off level > 10.6 (U/mL). Also, ***Bektafi et al.***[22] showed that Compared to the groups with adenomatous colon-rectal polyps, the CRC group had significantly higher levels of plasma M2-PK, with an AUC of 0.664, sensitivity of 35%, and specificity of 99.33%.

Unlike ***Fatela-Cantillo et al.***[23] who discovered that plasma M2-PK lacked the sensitivity and specificity to distinguish between benign and malignant colorectal diseases.

As regard CA19-9, we found no statistical significance between functional and organic groups as regard CA19-9 which disagreed with ***Hardt et al.***[24] which reported that it had sensitivity 33% in the diagnosis of CRC.

As regard stool analysis, there was high statistical significance between the functional and the organic groups as regard stool analysis with WBCs and RBCs in stool

were more increased in organic than functional colonic disorders. This agreed with ***Bastawy et al.***[21] which found the same results.

In our study, we found that there was high statistical significance between the functional and the organic groups as regard FOBT with sensitivity 51.7%, specificity 100% which agreed with ***Fu et al.***[25] with sensitivity 65%, specificity 93% and ***ELsafi et al.***[26] with sensitivity 65%, specificity 77.87%.

All of the patients in this investigation had highly significant positive associations between their plasma M2 PK level and their age, ESR, CRP, and CEA. Conversely, all patients had highly substantial negative associations between plasma M2 PK and Hb which agreed with ***Bastawy et al.***[21] which revealed that there was positive relation between fecal M2 PK and (age and ESR) and also significant negative relations between fecal M2 PK level and Hb.

Also, ***Wahib et al.***[20] found Higher sensitivity and specificity were obtained when plasma M2-PK and CEA showed a positive association. Moreover, ***Kumar et al.***[27] reported that combination between plasma M2PK and CEA increased sensitivity, PPV and NPV from 50, 83, 60% respectively to 67, 87 and 70% respectively in CRC patients while ***Meng et al.***[4] showed there is no significant link between CEA and M2-PK. Given that it was assessed in just 13 out of 153 CRC patients with a significant standard deviation, this could be related to the study's limited sample size.

There was a substantial statistical difference in our study between the endoscopic activity of ulcerative colitis and plasma M2-PK as plasma M2-PK was more increased in mayo1 & 2 & 3 (active disease) than Mayo 0 (normal or in active disease) which agreed with ***Wahib et al.***[20] which revealed that M2-PK was more elevated in mayo1 & 2 than mayo 0. also, ***Bastawy et al.***[21] revealed that M2-PK levels were significantly elevated in exacerbation than in remission of IBD.

In our study, there was high statistical significance in relation of plasma M2-PK to CRC histopathological severity as it was more increased in Mucinous adenocarcinoma than non - Mucinous adenocarcinoma. Also, there was statistical significance as regard plasma M2-PK to colo-rectal polyp histo-pathological severity as it was more increased in the adenomatous polyp (villous > tubulovillous > tubular) than non-adenomatous polyp (hyperplastic polyp). Also, there was no statistical significance as regard relation of plasma M2 - PK to IBD histopathology. this agreed with ***Meng et al.***[4] who reported that the plasma M2-PK level was 8.58, 6.70, 5.13, and 2.51 U/ml among advanced adenoma, adenomas, non - adenomatous polyps, and IBD, respectively.

The relationship between plasma M2-PK and colo-rectal polyp grading risk was highly statistically significant in this study because it was higher in adenomatous polyps (high risk adenoma > low risk adenoma) than non-adenomatous polyps (hyperplastic polyp). Additionally, a strong statistically significant difference was seen between the relationship between plasma M2-PK and the severity of colorectal cancer grading as it rose in (poorly differentiated > moderately differentiated > well differentiated).

This agreed with ***Hathurusinghe et al.***[28] and ***Fatela-Cantillo et al.***[23] who revealed that M2-PK was increased more in patients with distant metastasis than patients without metastasis. In contrast to ***Kumar et al.***[27] who revealed no significant correlation between M2-PK levels and tumor stage or differentiation.

Accordingly, the rate of compliance for screening of plasma M2-PK in organic colonic disorders is higher than CEA, FOBT, and colonoscopy. Because plasma M2-PK has good compliance, sensitivity, and specificity, it can be utilized as a screening test for organic colonic diseases.

Finally, we conclude that no single test is sufficient to diagnose organic colonic disorders. A plasma test is less invasive than a colonoscopy and is quicker, easier, and less expensive than a fecal test. So, It can be considered as a non-invasive bio-marker for early detection of organic colonic disorders.

CONCLUSIONS

Plasma M2-PK is increased in organic more than functional colonic disorders so, can be used to differentiate between them. Also, it can be considered a promising rapid, cheap non invasive bio-marker for early detection of organic colonic disorders.

Also, it can discriminate between benign and malignant colonic lesions. Also, it can differentiate between active

and inactive IBD. Also, it can evaluate grading risk of colo-rectal polyps and CRC.

In order to distinguish between various organic colonic illnesses and functional bowel disorders, more extensive research is required to assess plasma M2-PK.

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Competing interests

No conflicting interests are disclosed by the authors.

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تقييم ام ٢- بيروفات كينيز بالبلازما كدلالة بيولوجية حديثة فى التمييز بين أمراض القولون الوظيفية والعضوية

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الخلفية: قد يكون التمييز بين اضطرابات القولون الوظيفية والعضوية صعبا في بعض الاحيان لانها قد تظهر مع اعراض مشابهة الا اذا كانت اضطرابات القولون العضوية تظهر مع اعراض مثل النزيف الشرجي او فقر الدم او فقدان الوزن.. الخ لذا اجريت تجارب عديدة لاكتشاف فحوصات غير جراحية للتمييز بين اضطرابات القولون العضوية والوظيفيه.

الهدف من العمل: الاشارة الي الدور التشخيصي للام ٢ بيروفيت كاينيز كمؤشر حيوي للتمييز بين اضطرابات القولون الوظيفيه مثل القولون العصبي واضطرابات القولون العضوية مثل التهابات القولون المناعية واورام القولون الحميدة والخبيثة.

المرضي وطرق العمل: اجريت هذه الدراسة علي ٨٠ مريض تم تقسيمهم الي اربع مجموعات:

المجموعة الاولي: ٢٠ مريضا يعانون من اضطرابات القولون الوظيفية كمجموعة ضابطة.

المجموعة الثانية: ٢٠ مريض يعانون من امراض القولون الالتهابية.

المجموعة الثالثة: ٢٠ مريض يعانون من لحميات القولون.

المجموعة الرابعة: ٢٠ مريض يعانون من سرطان القولون.

النتائج: اظهرت هذه الدراسة انه يمكن استخدام تحليل ام ٢ بيروفيت كاينيز في التمييز بين بين اضطرابات القولون الوظيفيه والعضوية عند مستوي ٣ وحدة /مل مع حساسة ٩٣,٣ ٪ ونوعيه ١٠٠ ٪ كما يمكن استخدامه للتمييز بين لحميات القولون الحميدة واورام القولون الخبيثة عند مستوي ١٢ وحدة / مل مع حساسية ١٠٠ ٪ ونوعية ١٠٠ ٪ كما انه يوجد علاقة نوعية عالية المستوي بين ام ٢ بيروفيت كاينيز و دلالات اورام القولون الخبيثة.

الاستنتاج: يمكن استخدام مصل ام ٢ بيروفيت كاينيز للتمييز بين اضطرابات القولون الوظيفية والعضوية كما يمكن اعتباره علامة غير جراحية لفحص اضطرابات القولون العضوية المختلفة لتقليل التدخل بمنظار القولون غير الضرورى.