Diagnostic Value of Plasma M2-Pyruvate Kinase in Egyptian Patients with Colorectal Cancer

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Abstract:
Background: M2-pyruvate kinase (M2-PK) is a tumor growth key regulator and plays a crucial role in tumor metabolism and can dynamically regulate aerobic glycolsis. Notwithstanding, the colonoscopy is the gold standard for early detection of colorectal cancer (CRC), the acceptance of this invasive technique is low. **Aim of the work:** to assess the diagnostic value of plasma M2-PK in Egyptian patients with CRC. **Methods:** we measured the plasma M2-PK using Enzyme-linked immunosorbent assay in CRC patients and comparing these levels with those obtained from patients with functional bowel disorders (FBD), inflammatory bowel disease (IBD),colorectal polyp and apparently healthy individuals and to detect its sensitivity and specificity. **Results:** the value of plasma M2-PK level was higher in CRC patients, 15.22 U/ml than other groups. Average plasma M2-PK was 6.78, 5.28, 2.25 and 2.1 U/ml among colorectal polyp, IBD, FBD patients and normal individuals respectively. The plasma M2-PK can be used in differentiating between CRC and normal individual with 83.33% sensitivity, 100% specificity at area under the curve (AUC) 0.949 with cut-off >6U/ml. Also, it can be used to discriminate between colorectal polyp and CRC patients at cut-off level of >10.6 U/mL with 75.5% sensitivity and 87.5% specificity and to differentiate between FBD and organic colonic lesions at cut-off level >3 U/ml with 81.94% sensitivity and 83.3% specificity. **Conclusion:** plasma M2-PK was considerably elevated in CRC patients and may be used as non-invasive biomarker for CRC. **Recommendation:** future researches including a large and long-term follow-up studies are recommended.
Keywords: Colorectal cancer; M2-Pyruvate kinase; Functional bowel disorders; IBD; colorectal Polyp.

INTRODUCTION

Colorectal cancer (CRC) is a major public health problem, being the third most commonly diagnosed cancer and the fourth reason of cancer death worldwide [1].Most cases of CRC are preventable at early stage, and several randomized, controlled trials have shown lower mortality among patients who undergo screening than among those who do not [2,3]. Survival is markedly affected by the stage of the disease at the time of diagnosis [4].

Regardless of considerable progress that has been made over the past years in diagnosis and management strategies of CRC, still many of the CRC patients are diagnosed in late stages and do not respond to treatment [5], therefore studies should be done to improve our understanding of pathological process in the CRC for identifying the best diagnostic, prognostic, and predictive biomarkers supporting its role in prevention, early detection and treatment [6, 7].

In spite of the fact that, the gold standard for the early detection of CRC is colonoscopy; the acceptance of this invasive and expensive method is low even in more developed countries [8,9]. The selection of patients who should undergo colonoscopy and/or imaging procedures is one of the key points of the diagnostic process, which should avoid the abuse of invasive and costly tests as well as the underestimation of potentially harmful diseases. The investigation and procedures to differentiate organic from functional bowel disorders represents a considerable burden both for patients and community health service [10].

Premalignant conditions of CRC as chronic inflammatory bowel disease (IBD) and colorectal polyp are preventable. In all such cases, recognition of the disease at an early stage is essential to devise suitable preventive cancer strategies [11]. Therefore, to develop an alternative modality based on blood biomarkers

as the first line screening test is an alternative ideal way for the early detection of CRC [12].

There are four pyruvate kinase isoforms presenting in human. The M1 isoform is presented in most adult and differentiated tissues; L and R isoforms are expressed in liver and red blood cells; the dimeric form of the M2 isoform is a splice variant of M1 expressed in cancer cells and undifferentiated tissues [13]. M2-pyruvate kinase (M2-PK) play an important role in tumor metabolism and researches revealed that M2-PK may react as protein kinase and co-activator of transcription factors [14]. It is a tumor growth key regulator and can dynamically regulate glycolsis energy production and synthetic processes [2]. M2-PK in CRC patients was about 4 times higher than normal individuals [13]. Plasma M2-PK could be helpful in discriminating malignant and benign colorectal lesions and may yield insight in terms of survival [8].

AIM OF THE WORK:

The aim of the study is to determine the diagnostic value of plasma M2-PK in Egyptian patients with CRC.

PATIENTS AND METHODS

The study was conducted on 120 persons who fulfilling the designed inclusion criteria. The study was carried out in Tropical Medicine department, Al-Azhar University Hospitals (Al-Hussein and Sayed Galal Hospitals) during the period from June 2015 to June 2018.

Inclusion criteria:

• Age ≥18 years.
• Patient with lower GIT symptoms and have an indication for lower endoscoy including chronic constipation, chronic diarrhea, changing in bowel habits and bleeding per rectum and other relevant alarming symptoms and signs e.g. significant unexplained weight loss, unexplained anemia, patients with remote metastases proved to be adenocarcinoma and were suspected to have CRC and patients underwent screening for CRC.
• The inclusion criteria for FBD group: confirmed diagnosis of FBD after full work up (Endoscopies, histopathology, etc).
• The inclusion criteria for the IBD patients: confirmed diagnosis of IBD was ascertained using conventional clinical, endoscopic, radiological and histopathological criteria.

• The inclusion criteria for colorectal polyp group: confirmed diagnosis by Endoscopies and histopathology other than polyp of IBD and CRC.
• The inclusion criteria for CRC group: confirmed diagnosis by Endoscopies and histopathology.

Exclusion criteria:

☒ Patients who refused to participate in the study.
☒ Patients who previously underwent surgery for CRC or received chemotherapy for CRC.
☒ Patients having cancer at any other site.
☒ Sepsis.

All the studied patients were subjected to: Full history taking, thorough clinical examination, imaging, colonoscopy and biopsies and routine laboratory investigations and specific tumor marker; Carcinoembryonic antigen (CEA) and plasma M2-PK.

Measurement of plasma M2-PK:

Blood was drawn from each patient into EDTA containing plasma tubes, centrifuged at the speed of 2000-3000 rpm for 20-min. Supernatant was removed, if precipitation appeared, centrifuged again. All icteric or hemolytic blood samples discarded. Plasma samples were stored at -80°C until the assay was performed. Our assay carefully followed the instructions of the test kit.

The study was done according to the Ethical Board of Al-Azhar University. All subjects involved in the current study were informed about the nature and details of the work and a written consent was obtained for each participant.

One hundred twenty individuals were involved in this study and classified into five groups:

Group I: Including 24 healthy persons.
Group II: Including 24 patients with FBD (Irritable bowel syndrome: 22; functional diarrhea: 1 and functional constipation: 1).
Group III: Including 24 patients with IBD (Ulcerative colitis (UC): 23 and Crhon's Disease (CD): 1).
Group IV: Including 24 patients with colorectal polyp (adenomatous polyp: 21; non-adenomatous polyp: 3).
Group V: Including 24 patients with CRC.

Statistical analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version (23) for statistical analysis. Quantitative data were expressed as

mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. The comparison between groups regarding qualitative data was done by using Chi-square test. The comparison between more than two independent groups with quantitative data and parametric distribution was done by using One Way ANOVA test with post hoc analysis. Also Receiver operating characteristic curve (ROC) were used to assess the best cut-off point with sensitivity, specificity, positive and negative predictive value and area under curve (AUC). The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at the level of < 0.05 and p-value <0.001 was considered as highly significant.

RESULTS

Table 1 shows the demographic features of the study population. The age of FBD group was 41.80 ± 10.82, in IBD patient's 34.54 ± 11.43, in colorectal polyp patient 45.08 ± 11.80 in and CRC patients, 50.38 ±11.26 years. There was no statistical significant difference between studied groups as regard gender. However, in IBD group females (58.3%) are more common than males while males are more common in colorectal polyp and cancer groups62.5% and 58.3% respectively. The main presentations in our CRC patients were bleeding per rectum experienced by (33.3%), followed by change of bowel habits about (29.2%) then weight loss (20.8%).

Regarding ESR, there was a significant difference between normal and FBD groups and patients with organic colonic lesions (IBD, colorectal polyp & cancer). Also, CRP show a significant difference between the studied groups mainly elevated in IBD group as shown in table 2. According to the anatomic site of CRC the current study showed that the rectal cancer (37.5%) was more common than proximal (33.33%) and distal colon cancer (29.17%). The present study showed that conventional adenocarcinoma (87.5%) was more common than mucinous adenocarcinoma (12.5%). Based on histologic differentiation the current study showed that the most frequent grading of the tumor was (66.7%) moderately differentiated, followed by poorly differentiated (20%) then well differentiated (12.5%). Lymph nodes involvement in CRC patient was 58.3% while, evidence of distant metastasis was 16.7%. Most of the patient

presented at stage III (37.5%) and stage II (33.3%) followed by stage IV (20.8%) and only 8.3% of patients presented at stage I.

Table 2 shows average of tumor marker of the studied groups. The mean value of CEA in normal and benign groups was nearly below 5 ng/mL while, in CRC group its mean was 88.4 ng/ml and median53ng/ml. CEA was higher in CRC group than other groups, with low sensitivity (66.67%) at AUC 0.711 when compared to normal population (table 3).

The value of plasma M2-PK level was higher in CRC patients15.22±8 U/ml than other groups (p-value <0.001). Average plasma M2-PK was 6.78±3.48, 5.28±3.33, 2.25± and 2.1±1.75 U/ml among colorectal polyp, IBD, FBD patients and normal individuals respectively (table 2). The value of plasma M2-PK level was higher in active UC 8.2±4.7 compared to inactive UC patients 3.64±2.3 U/ml. While, there was no statically significant difference as regard relation of M2-PK to colorectal polyp risk however, it more elevated in high risk adenoma than non-adenomatous polyp. There was statically significant difference as regard relation of M2-PK to evidence of distant metastasis while there was no statically significant difference regarding sex, site, histological type, lymph node involvement and stage (table 4). Also, no statistical significant correlations between plasma M2 PK and age in CRC group (r=0.06; P=0.8).

The performed analysis also focused on the determination of a cut-off for CRC prediction. This optimal cut-off was estimated at >6U/mL at AUC 0.949 with 83.33% sensitivity (missing rate was 16.67%), 100% specificity, 100% PPV and 98.2% NPV (table 3). These results support the role of plasma M2-PK in differentiating between CRC and normal individual. Also, plasma M2-PK can be used to discriminate between polyp group and CRC group at AUC 0.827 with a cut-off level of >10.6 U/ml, with 75.5% sensitivity, 87.5% specificity, 40.0% PPV and 96.9% NPV (table 5). Also, our study showed that plasma M2-PK can be used to discriminate between to discriminate FBD from organic colonic lesion at AUC 0.872 with a cut-off level of >3 U/mL, with 81.94% sensitivity, 83.3% specificity, 35.3% PPV and 97.6% NPV (table 6). In our study we found positive correlation in direct proportion between plasma M2-PK and CEA (r=0.50; P< 0.001) and

combination can give higher sensitivity and
specificity.

Tables (1): Demographic features of the studied groups

Groups Variables		Group I (n = 24)	Group II (n = 24)	Group III (n = 24)	Group IV (n = 24)	Group V (n = 24)	P-value
Age (years)	Mean	44.83	41.88	34.54	45.08	50.38	< **0.001***
	±SD	11.87	10.82	11.43	11.80	11.26	
	Range	22-66	20-59	18-55	18-65	24-69	
Gender	Male	12 (50%)	13 (54.2%)	10 (41.7%)	15 (62.5%)	14 (58.3%)	0.6
	Female	12 (50%)	11 (45.8%)	14 (58.3%)	9 (37.5%)	10 (41.7%)	

*: p-value < 0.001is considered highly significant.

Table (2): Comparison between studied groups as regard ESR and CRP and tumor markers

Variables	Groups	Group I (n = 24)	Group II (n = 24)	Group III (n = 24)	Group IV (n = 24)	Group V (n = 24)	P-value
ESR (mm)	Mean	6.83	8.04	12.38	17.75	29.21	**< 0.001***
	±SD	1.87	1.45	2.86	2.16	3.72	
CRP (mg/dl)	Mean	3.38	3.71	8.29	4.54	5.00	**0.003****
	±SD	0.44	0.33	1.66	1.11	1.21	
CEA ng/mL	Mean	2.09	2.31	5.28	4.93	88.4	**<0.001***
	±SD	0.72	0.03	1.33	1.61	17.96	
M2-PK U/mL	Mean	2.1	2.25	5.28	6.78	15.22	**< 0.001***
	±SD	0.75	0.47	1.33	1.48	2.00	

*: p-value < 0.001is considered highly significant; **: p-value < 0.05 is considered significant
ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.

Table (3): Diagnostic performance of Plasma M2-PK and serum CEA in discrimination of normal group and CRC group

	Cut-off	Area under the curve	Sensitivity	Specificity	PPV	NPV	p-value
M2-PK	> 6 U/mL	0.949	83.33%	100 %	100 %	98.2%	< 0.001*
CEA	> 7 ng/mL	0.711	66.67%	100 %	100 %	96.4%	0.0188**

*: p-value < 0.001is considered highly significant; **: p-value < 0.05 is considered significant
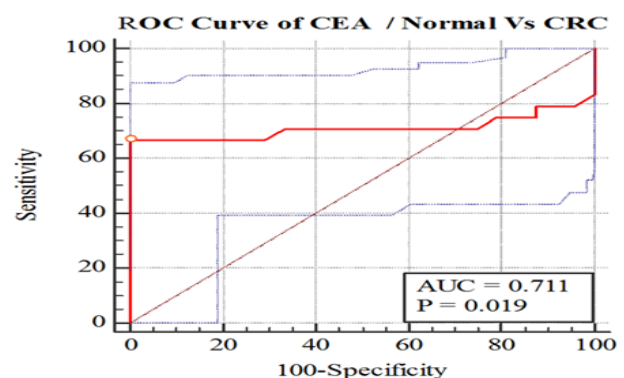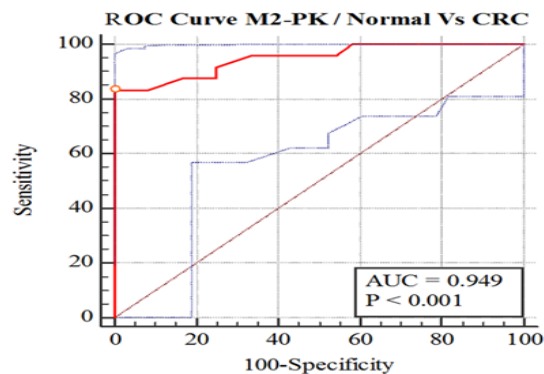
Figure (1): ROC curve between control group and CRC group as regard plasma M2-PK and serum CEA

Table (4): Relation of M2-PK to different prognostic factor in CRC patients

Variant		M2-PK Mean±SD	P-value
Sex	**Male (n=14)**	15.4±7.6	P = 0.802
	Female (n=10)	16.4±8.4	
Site of CRC	**Proximal (n=8)**	12.8±7.85	P = 0.522
	Distal (n=7)	15.21±6.43	
	Rectum (n=9)	17.37±9.39	
Histopathological type of CRC	**Adenocarcinoma(n=21)**	14.54±7.92	P = 0.281
	Mucinous adenocarcinoma(n=3)	19.97±8.36	
Lymph node involvement	**No (n=10)**	12.13±6.15	P = 0.112
	Yes (n=14)	17.42±8.63	
Evidence of distant metastasis	**No (n=10)**	13.76±7.54	*P = 0.043
	Yes (n=4)	22.50±6.75	
Stage	**Stage I & II (n=10)**	14.16±8.87	P = 0.596
	Stage III & IV(n=14)	15.97±7.57	

*: p-value < 0.05 is considered significant

Table (5): Diagnostic performance of Plasma M2-PK and serum CEA in discrimination of polyp group and CRC group

	Cut-off	Area under the curve	Sensitivity	Specificity	PPV	NPV	p-value
M2-PK	> 10.6U/mL	0.827	75.0 %	87.5 %	40.0%	96.9%	< 0.001*
CEA	> 10.7 ng/mL	0.727	66.67 %	95.83%	64.0%	96.3%	0.0071**

*: p-value < 0.001is considered highly significant;**: p-value < 0.05 is considered significant
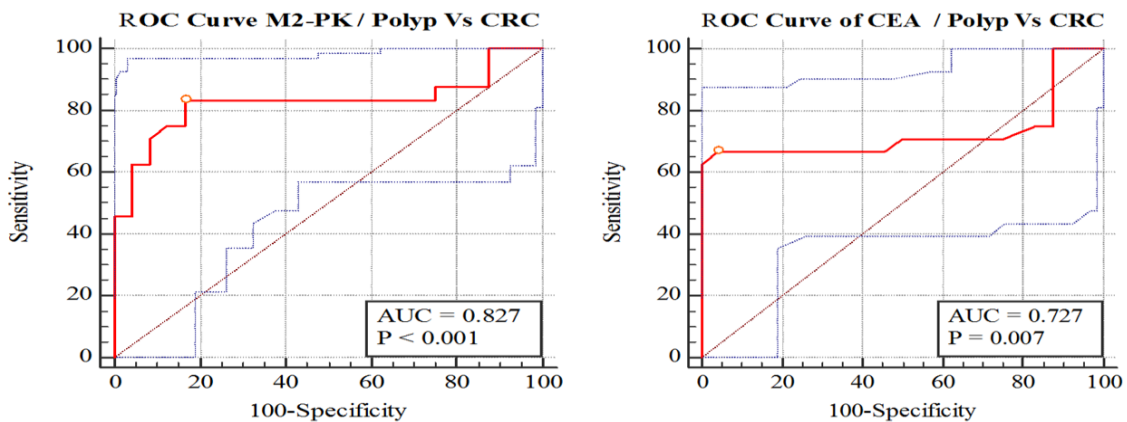

Figure (2): ROC curve between polyp group and CRC group as regard plasma M2-PK & serum CEA

Table (6): Diagnostic performance of Plasma M2-PK in discrimination of FBD from organic colonic lesions

	Cut-off	Area under the curve	Sensitivity	Specificity	PPV	NPV	p-value
M2-PK	> 3(U/mL)	0.872	81.94 %	83.33 %	35.3%	97.6%	< 0.001*

*: p-value < 0.001is considered highly significant.

Discussion

Although, the recent advances in multidisciplinary management and treatment of CRC, over the last years, no single test is currently able to diagnose. Patients are still reluctant to undergo screening tests because of the associated invasiveness and discomfort [16]. The ideal biomarker for CRC has not been found [17]. So, there is a critical need for reliable, minimally invasive, highly sensitive and specific markers of an individualized and optimized patient treatment at the earliest disease stage as possible [18].

Our aim in the current study was to investigate the potential value of the plasma M2-PK in Egyptian patients with CRC and other colorectal lesions and compared its value to that of serum CEA to detect its sensitivity and specificity as a non-invasive biomarker in identification of such patients. We measured enzyme levels in EDTA-plasma samples as EDTA-plasma is the most appropriate sample for obtaining the diagnosis of this tumor marker [19]. Tumor M2-PK can also be detected and measured in stool samples using an ELISA [20]. It was shown that fecal M2-PK is more accurate for CRC screening than plasma or serum tumor M2-PK [21]. However, blood tests are likely to be more acceptable than stool tests in population-based screening [22].

The present study showed that there was highly statistical significant difference (p-value <0.001) as regard plasma M2-PK, in CRC groups compared to other groups in all colorectal lesions. The mean plasma M2-PK level for CRC patients was 15.22± 8 U/ml. The plasma M2-PK can be used in differentiating between CRC and normal individual with 83.33% sensitivity, 100% specificity at (AUC) 0.949 with cut-off >6 U/ml higher than that of CEA. Our results are in agreement with the study of **Meng et al.**[13] which found significantly higher serum M2-PK in CRC patients compared to adenoma, non-adenomatous polyps, IBD and normal population with higher 94.62 % sensitivity and low 55.06% specificity at cut-off 2.5 U/ml. **Hathurusinghe et al.**[23] reported in his a systematic review of an eight article that specificities were 89% or greater, sensitivity ranged from 47.8% to 76.5%. Also, **Bektafi et al.**[8] in his study found that the CRC group had significantly higher levels of plasma M2-PK than the groups with adenomatous colorectal polyps and AUC was 0.664 the sensitivity was 35% and specificity was 99.33%.Although, our performance is superior, but our study was carried on a small sample size (n=24) beside the different best cut-off point and laboratory methods.

The current study showed that there was no statically significant difference as regard relation of M2-PK to age, sex, site, histopathological type, lymph node involvement and stage of CRC while, there was statically significant difference as regard relation of M2-PK to evidence of distant metastasis. Likely, **Hathurusingh et al.**[23] found that patients with distant metastasis had higher levels of tumor M2-PK than patients without metastasis. Also, Fatela-Cantillo **et al.**[24] supported this issue by finding that plasma M2-PK levels were significantly higher in patients with distant metastases and advanced stage than other which, indicated that plasma M2-PK testing on admission may have a potential prognostic role in identifying individuals with metastatic CRC and at an advanced stage. While, there was no relation to site of CRC and histopathological type of CRC. Also, **Fatela-Cantillo et al.**[24] suggested that plasmatic M2-PK may be a predictor of death risk when its plasma levels more than 20 U/mL.

In contrast, **Kumar*et al.*[25]** could not find an association betweenM2-PK levels and advanced tumor stage and differentiation.

Munoz-Colmenero*et al.*[26] in their study compared plasma tumor M2-PK levels in different cancer types. Nevertheless, various studies have demonstrated the increase of plasma M2-PK in different cancer (low specificity), the published literature is poor, only limited data exist on plasma tumor M2-PK in different cancer types such as breast, renal, lung, pancreatic and cervical cancer. We evaluated this marker in patient with colonic lesions and exclude any cancer other than CRC from the start and also we evaluate its relation to different prognostic factors and in differentiation of benign from malignant colonic lesions.

The current study showed that Plasma M2-PK can be used to discriminate between benign from malignant colonic lesion at a cut-off level of >10 U/mL, with 83.33% sensitivity, 87.5% specificity, 42.6% PPV and 97.9% NPV.

Likely, **Meng *et al.*[13] and Bektafi *et al.*[8]** reported that plasma M2-PK can differentiate between benign and malignant colonic lesion. Unlikely, **Fatela-Cantillo *et al.*[24]** stated that there is still no evidence that plasma M2-PK is sufficiently sensitive or specific to justify using it to differentiate malignant from benign colorectal disease for an individual patient.

The current study showed that plasma M2-PK can be used to discriminate between to discriminate FBD from organic colonic lesion at a cut-off level of >3U/mL, with 81.94% sensitivity, 83.3% specificity, 35.3% PPV and 97.6% NPV**.** Furthermore, the results of M2-PK levels in our study were shown to be significantly elevated in Myo endosopic score (1 & 2), compared to normal or inactive UC.

Similarly, **Bastawy *et al.*[27]** revealed a highly significant increase in tumor M2-PK in the stool samples of patients with organic colonic disorders (IBD and CRC groups) compared to functional group (IBS). At a cut-off value of 4.2 (U/ml), the overall sensitivity and specificity for organic group over the functional group were 87.5% and 80% respectively. Additionally, the results of M2-PK levels in his study were shown to be significantly elevated in active, compared to inactive IBD.

Data from **Almousa *et al.*[28]** suggested that blood PKM2 as a promising biomarker for IBD and the dysbiosis of microflora in CD. Also,

Marin *et al.*[29] showed that serum PKM2 levels appear to be a reliable biomarker of IBD activity. Likely, Chung-Faye *et al.*[30] in their study found high levels of M2-PK were documented in 81 adults diagnosed with IBD. This cohort was compared to a group of 43 subjects with IBS and 7 with CRC. M2-PK concentrations were higher in patients with IBD than in the controls. Furthermore, higher levels were evident in individuals with active IBD than in those with quiescent disease. In a further study by **Jeffery *et al.*[31]** M2-PK was assessed in 105 adults presenting with undifferentiated gastrointestinal symptoms and 94 healthy controls. The 14 adults subsequently diagnosed with organic diseases (only 10 with IBD) had higher fecal concentrations of M2-PK than those with functional symptoms or the controls. M2-PK measurement provided sensitivity of 67% and specificity of 88% in distinguishing between organic and functional diagnoses. However, it had a lower sensitivity, specificity and predictive value than fecal calprotectin in differentiation of organic disease from functional bowel disorders. However, this is in contradiction of the observation of **Roszak *et al.*[32]** who stated that M2-PK is a more sensitive marker than calprotectin and lactoferrin in evaluating disease activity in UC or CD.

In our study we find positive correlation in direct proportion between plasma M2-PK and CEA (r=0.50; P< 0.001) and combination can give higher sensitivity and specificity.

In contrast, **Meng *et al.*[13]** did not find a correlation between M2-PK and CEA levels r=0.37, p=0.26). This may be due to the limited number of patients in his study as CEA levels measured in only 13 patients as well as due to a high standard deviation.

Dowling *et al.*[33] in their study established that the level of several biomolecules, including M2P-K,serotonin, gamma enolase and members of the 14-3-3 family of proteins, showed statistically significant changes when comparing CRC patients with non-malignant patients. Also, Fung *et al.*[34] identified a panel of three biomarkers (M2-PK, Insulin like growth factor binding protein 2 (IGFBP2), Dickkopf-, 3 and (DKK3) that discriminated between controls and CRC with 73% sensitivity at 95% specificity, or early stage (Stage I and - II), raising the possibility for its use as a non-invasive blood diagnostic or screening test.

Conclusion

Plasma M2-PKlevels were significantly elevated in patients with CRC and may be helpful in distinguishing such patients from healthy subjects or patient with other colonic lesions. This suggests that the plasma M2-PK is fast non-invasive biomarker for CRC detection. Also, plasma M2-PK can differentiate between functional and organic colonic lesions.

Recommendation

Future researches including a large and long-term follow-up studies are recommended. Combined studies of different markers with plasma M2-PK either fecal or blood are recommended to increase the efficacy in diagnosis of CRC.

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