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Evaluation of Fecal Pyruvatekinase Isoenzyme (M2-Pk) Level in Differentiating Functional from Organic Colonic Disorders

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Abstract

Background: Pyruvatekinase, which converts phosphoenolpyruvate to pyruvate, is a key enzyme in glucose metabolism and is present in organ-specific isoforms (the L, R, M1, and M2 isoforms). In normal proliferating cells, M2-PK is mainly tetrameric and has a high affinity for phosphoenolpyruvate. In contrast, the M2-PK isoenzyme found in tumor cells is usually dimeric and has a low affinity for phosphoenolpyruvate. For this reason, the dimeric form of M2-PK has been named tumor M2-PK.
Objective: This study evaluated the potential value of fecal, dimeric M2-PK level in differentiating functional from organic colonic disorders as well as its value as a surrogate marker of inflammation in patients with inflammatory bowel disease (IBD) and colorectal cancer (CRC).
Patients and Methods: This prospective study included 60 patients with different colonic disorders, 20 patients with Functional colonic disorders, 20 patients with inflammatory bowel disease (ulcerative colitis & chron's disease) and 20 patients with colon cancer. The M2-PK level was measured in all patients using a highly sensitive enzyme – linked immunosorbent assay (ELISA), which allowed the quantitative measurement of tumor M2-PK in stool.
Results: Our study revealed a highly significant increase in tumor M2-PK in the stool samples of those patients with organic colonic disorders (IBD and CRC groups) compared to functional group (IBS). At a cut-off value of 4.2 (U/ml), our overall sensitivity and specificity for organic group over the functional group were 87.5% and 80% respectively. Furthermore, the results of M2-PK levels (U/mL) in our study were shown to be significantly elevated in active, compared to inactive IBD.
Conclusion: In colonic disorders, fecal concentration of tumor M2-PK is a good marker for discrimination of functional from organic colonic conditions (IBD and CRC), with a sensitivity and specificity of **87.5% and 80%** respectively. Tumor M2-PK can be used as a tumor marker in screening of colorectal cancer. Dimeric fecal M2-PK has the potential to be an important noninvasive marker of disease activity in IBD.

Keywords: M2-pyruvate kinase, inflammatory bowel disease, Crohn's disease, ulcerative colitis, colorectal cancer, irritable bowel syndrome.

Introduction

Differentiating between inflammatory bowel disease (IBD) and functional bowel disorders, such as irritable bowel syndrome (IBS), can often be difficult as they present with similar symptoms ***(Kenneth, 2011).***

The diagnosis of IBD typically necessitates invasive endoscopic procedures to visualize the mucosa and enable confirmatory histological specimens to be obtained. However, this may miss disease in the gastrointestinal (GI) tract, not directly visualized at endoscopy. Furthermore, in IBD accurate monitoring of disease activity may include repeated endoscopy, as symptoms correlate poorly with disease activity ***(Sutherland et al., 2008).***

Noninvasive biomarkers in IBD are being increasingly recognized as important, both at the initial diagnosis and for monitoring disease activity. They also play a valuable role in differentiating organic GI disease from functional disorders by examining the entire GI tract ***(Däbritz et al., 2014).***

Key characteristics of fecal biomarkers include stability in fecal samples and the existence of a sensitive and reliable assay ***(Christofk et al., 2008).***

Pyruvatekinase, which converts phosphoenolpyruvate to pyruvate, is a key enzyme in glucose metabolism and is present in organ-specific isoforms (the L, R, M1, and M2 isoforms). In normal proliferating cells, M2-PK is mainly tetrameric and has a high affinity for phosphoenolpyruvate. In contrast, the M2PK isoenzyme found in tumor cells is usually dimeric and has a low affinity for phosphoenolpyruvate. Dissociation of the tetrameric form to the dimeric form in tumor cells is induced by direct interaction of M2-PK with various oncoproteins. For this reason, the dimeric form of M2PK has been named tumor M2PK. Because of its low affinity for phosphoenolpyruvate, tumor M2-PK is easily released from tumor cells and is quantitatively detectable in body fluids ***(Abdullah et al., 2012).***

Tumor M2-PK can also be detected and quantified in stool samples using an ELISA. It was shown that fecal tumor M2PK is more accurate for CRC screening than serum or plasma tumor M2PK ***(Tonus et al., 2012).*** Abdullah et al. showed that the fecal tumor M2PK test has good sensitivity and specificity for CRC detection, especially in high-risk or symptomatic populations ***(Abdullah et al., 2012).***

M2-PK is crucial for rapid tumor growth and aerobic glycolysis during tumorigenesis ***(Christofk et al., 2008).*** Its tetrameric and dimeric forms are present in rapidly proliferating cells of many tissues, including leukocytes. Upon leukocyte destruction in the gastrointestinal tract, the protein is released to the fecal stream ***(Gupta and Bamezai, 2010).***

M2-PK is stable in stools, which increases the potential value of fecal M2-PK concentration assessment both in intestinal inflammation and cancer. They postulated that fecal M2-PK could serve as a biomarker of inflammation in inflammatory bowel diseases (IBD) ***(Czub et al., 2007).***

Active IBD is accompanied by increased cell turnover and rapid division ***(Foell et al., 2009).*** Turner et al. investigated fecal M2-PK, FC, lactoferrin and S100A12 protein in children with severe ulcerative colitis. They showed that only M2-PK had constructive and predictive validity, while other markers failed to meet this criterion ***(Turner et al., 2010).***

The present work aimed to evaluate the potential value of fecal, dimeric M2-PK level in differentiating functional from organic colonic disorders as well as its value as a surrogate marker of inflammation in patients with inflammatory bowel disease (IBD) and colorectal cancer (CRC).

Patients and Methods

Patients attending the gastroenterology outpatient clinic and in patient Department of Gastroenterology and Oncosurgery, El Sayed Galal teaching hospital, during the period from August 2014 to August 2016, with new-onset lower GI symptoms, with previously diagnosed IBD or with previously diagnosed CRC were prospectively enrolled in the study. Patients with symptoms of dyspepsia or gastroesophageal reflux disease were excluded. All patients underwent clinical evaluation, including history (including medication) and full physical examination before providing a fecal sample for M2-PK assays. Patients presenting with diarrhea also provided a stool samples for microscopy (ova, cysts, and parasites). Further investigations, including endoscopic examinations, were enrolled as clinically indicated. For the purposes of this analysis, patients were subdivided into the following diagnostic categories; IBD group (ulcerative colitis (UC) and Crohn's disease (CD), irritable bowel syndrome/functional bowel disorder (IBS), and colorectal cancer (CA). In a subanalysis of IBD patients, disease activity was assessed by a combination of physician global assessment, and/or endoscopic grading where available ***(D'Haens et al., 2007).*** Histopathological examinations of colonic biopsies were done.

Patients provided a single stool sample for analysis, submitted within 48 hours. For M2-PK, a commercially available ELISA kit (ScheboTech, Giessen, Germany), which has no crossreactivity to other forms of pyruvate kinase was used ***(Tonus et al., 2012)***. Patients did not have to keep a special diet and were told to take their usual medications. Usually 10mg feces samples were collected by sterile tube. This assay has an intratest and interest variability coefficient of 4.5% and 6.1%, respectively. Stool samples for M2-PK are stable at room temperature (21°C) for 3 days or for up to 1 year at -20°C ***(Tonus et al., 2012).***

This study was approved by Al-Azhar Faculty of Medicine Local Research Ethics Committee. The protocol was explained to at least one relative of each patient selected for the study, and written informed consent was obtained from all patients who participated or from their relatives.

Statistical analysis

The data were analyzed using Microsoft Excel 2010 and statistical package for social science (SPSS version 24.0) for windows (SPSS IBM., Chicago, IL).

Results was expressed as mean ± SE with 95% confidence interval using mean for quantitative variables, and using the frequencies and percentage for qualitative ones; a P value **< 0.05** considered statistically significant.

Quantitative data were analyzed by applying one way analysis of variance (ANOVA) test for comparison of the mean of more than two groups, while Student t-test was used for comparison of the mean of two groups.
The ROC curve was used to detect the cutoff points and the correlation between the sensitivity and specificity of functional, IBD and CRC groups.

Results

Of the 60 patients evaluated, 18 had biopsy-proven UC, 2 had biopsy-proven CD and 20 had biopsy-proven CRC. In all, 20 had normal investigations and were diagnosed as having functional bowel disorders/IBS after full assessment.

In the functional bowel disorder subgroup, the vast majority of patients had IBS according to ROME III criteria. However, in all these patients all clinically indicated endoscopic investigations were normal. The main diagnostic groups are summarized in **(Table 1).** Student t-test was positive for age between IBS group and each one of the other diagnostic groups, indicating a significant difference.

Table (1): Age and Gender in the Main Diagnostic Groups

Groups	N	M:F Ratio	Age (yrs)	P for age* (vs. IBS)
Colorectal cancer	20	14:6	40-79	0.03
Inflammatory bowel disease	20	6:14	18 – 57	0.03
IBS/functional bowel	20	10:10	21 - 55	-
Total	60	30:30		-

Table (2): Fecal M2PK Values in the Main Diagnostic Groups

Fecal M2PK (U/mL) Group	N	Min	Max	Mean	S.E	P. Value	
						a	b
IBS/functional bowel	20	1.20	29.0	5.18	1.5		
Inflammatory bowel disease	20	3.0	279.0	49.9	15.5	0.01*	
Colorectal cancer	20	1.6	217.0	62.5	14.3	0.001**	0.5

[a]p= Another groups compared with Functional group.
[b]p= CRC group compared with IBD group.

Kruskal–Wallis ANOVA was positive for M2-PK between the 3 diagnostic groups, indicating a significant difference between the groups. The mean values of M2-PK were highly significantly elevated in IBD and CRC patients compared to IBS**.** Although M2-PK concentrations in IBD patients were higher than the CRC group; these differences were not statistically significant (p value **= 0.5) (Table 2).**

There were highly significant differences in the M2-PK concentrations in patients with active versus inactive disease **(Table 3).**

Table (3): Fecal M2PK Values in Active vs. Inactive IBD disease

Fecal M2PK (U/mL) Group		N	Min	Max	Mean	S.E	P. Value
IBD	In remission	10	7.0	35.0	16.7	2.6	0.02*
	In exacerbation	10	3.0	279.0	83.3	27.6	

Fecal M2-PK had a sensitivity of 87.5%, specificity of 80%, PPV of 89.7%, and a negative predictive value (NPV) of 76.2% for organic GI diseases. Similar results were obtained when the analyses were performed for IBD alone (sensitivity of 95%, specificity of 80%, PPV of 82%, and a negative predictive value (NPV) of 94%), and for colorectal cancer patients alone (sensitivity of 87.5%, specificity of 80%, PPV of 89.7%, and a negative predictive value (NPV) of 76.2% **(Fig. 1 and Table 4).**

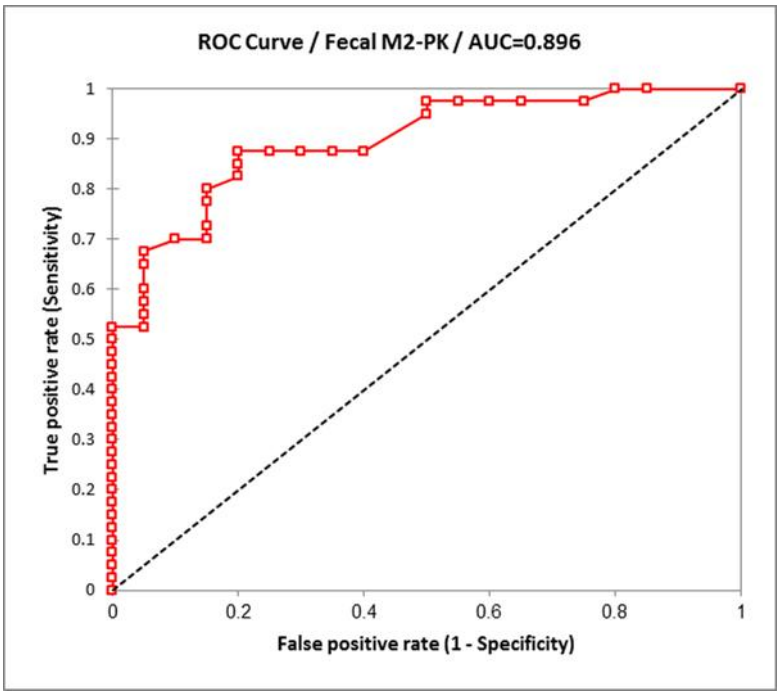

Fig. (1): Receiver operator characteristic (ROC) plot for Fecal M2-PK between Functional and Organic groups (area under the curve AUC = 0.896)

Table (4): Sensitivity, specificity, efficacy, positive and negative predictive values of the plasma concentrations of tumer M2-PK for Organic GI Disease

Cutoff	Sensitivity	Specificity	PPV	NPV	TP	TN	FP	FN	Accuracy	P. Value
4.2	87.5	80.0	89.7	76.2	35	16	4	5	85.0	0.001**

Discussion

The gold standard investigation for the early detection of colorectal cancer is colonoscopy. However, the acceptance of this costly and invasive method is low ***(Mariann et al., 2016).***

In order to increase the participation in colorectal cancer screening programs, an easy, fast and economical initial screening method, with good patient compliance, is absolutely necessary. This allows identification of those patients most likely to have colorectal cancer, who require further investigation by colonoscopy.

An increase in tumor M2-PK in EDTA plasma samples is found in gastrointestinal cancers, as well as a wide range of other tumors such as lung, renal, breast and cervical cancer. Thus, fecal tumor M2PK is more accurate for CRC screening than serum or plasma tumor M2PK ***(Tonus et al., 2012).***

Our study revealed a highly significant increase in tumor M2-PK in the stool samples of those patients with organic colonic disorders (IBD and CRC groups) compared to functional group (IBS). At a cut-off value of **4.2** (U/ml) our overall sensitivity and specificity for organic group over the functional group were 87.5% and 80% respectively. These data corresponded well with ***Chung-Faye et al. (2007)*** who found that mean M2-PK values were significantly elevated in UC, CD, and colorectal carcinoma compared to IBS. Using a predetermined cut-off level for normal fecal M2-PK, a sensitivity, specificity, and positive predictive value of 73%, 74%, and 89%, respectively, for differentiating organic disease from IBS were obtained.

These data also corresponded well with ***Mariann et al., (2016)*** who found that sensitivity and specificity of M2-PK for CRC were 94.7% and 67.5%.

Studies suggest that inflammatory reactions in the bowel can cause an elevation in fecal tumor M2PK level ***(Mulder et al., 2007 and Shastri et al., 2008).*** In our study, 95% of patients with IBD had positive M2PK test results at cutoff value of **4.2** U/ml. Most IBD patients with positive results had ulcerative colitis IBD.

The results of the present study showed significant differences in the level of fecal tumor M2-PK between IBD cases and functional group (IBS) cases, while the results revealed a non-significant difference between IBD and CRC groups. Of the 20 patients evaluated with IBD, 18 had biopsy-proven ulcerative colitis and 2 had biopsy-proven crohn's disease. The mean tumor M2-PK value in IBD group was 49.9 U/ul, and at a cut-off value of **4.2** U/ml, our overall sensitivity for IBD was **95 %,** and its specificity was **80 %**. These data came in correlation with the results of a study done by ***Mulder et al. (2007)*** who showed that in IBD patients, the M2-PK test was positive in (78.9%). In another multicenter study done by ***Shastri et al. (2008)***, up to **88%** of patients with ulcerative colitis and Crohn's Disease had elevated M2-PK levels. Furthermore, the results of M2-PK levels (U/mL) in our study were shown to be significantly elevated in active, compared to inactive, IBD and these data came in correlation with ***Chung-Faye et al. (2007).***

In our study, the functional group consisted of 20 individuals with ROME III citeria for IBS. The mean tumor M2-PK value in this group was 5.18 U/ml. In 16 of 20 subjects, tumor M2-PK levels were below the cut-off value. The resulting specificity at a cut off value of 4.2 U/ml was 80 %, which is in general accordance with the study of ***Koss et al. (2008)*** who reported specificities between 50% and 98***%.*** Positive results in patients with normal colonoscopy results may have been caused by inflammation, which have been detected by microscopic examination. ***Shastri et al. (2008)*** also showed that 26.2% of normal subjects, including patients with irritable bowel syndrome, (135/156; cutoff value >4 U/mL) had positive fecal tumor M2-PK test results. By using a combination of clinical factors, such as age, symptoms (especially those fulfilling ROME II criteria), and noninvasive fecal markers, a significant proportion of patients with inactive IBD or IBS may avoid further invasive endoscopic investigations, reserving endoscopy only for those with elevated fecal M2-PK concentrations.

Cancer cells have higher rates of glucose uptake than normal cells. However, only a small fraction of the glucose taken up is used for oxidative phosphorylation. The decrease in aerobic glycolysis in these cells may be due to reprogramming of metabolic genes, enabling cancer cells to partition glucose metabolites away from oxidation towards the synthesis of macromolecules ***(Uppara et al., 2015).*** M2-PK is crucial for rapid tumor growth and aerobic glycolysis during tumorigenesis ***(Christofk et al., 2008).*** Therefore, M2-PK can be used as a noninvasive biomarker and a diagnostic tool for the detection of cancer.

Conclusion

Fecal concentration of tumor M2-PK is a good marker for discrimination of functional from organic colonic conditions (IBD and CRC); with a sensitivity and specificity of **87.5% and 80%** respectively. Tumor M2-PK can be used as a

tumor marker in screening of colorectal cancer. Dimeric fecal M2-PK has the potential to be an important noninvasive marker of disease activity in IBD.

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