Original Article

Diagnostic Value of Pyruvate Kinase Isoenzyme Type M2 in Colon Cancer Proven with Colonoscopy

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Abstract

Background: Colonoscopy is the gold standard for colon cancer screening; it is also associated with a high cost and complication. Proliferating cells, in particular tumor cells, express a dimeric isoenzyme of pyruvate kinase, termed M2 pyruvate kinase (M2-PK). The aim of this study was to determine the diagnostic accuracy of fecal M2-PK for colon cancer. **Materials and Methods:** Forty-nine patients with colon cancers and 49 healthy controls were selected consecutively among individuals undergoing screening colonoscopy for various indications. The diagnosis was confirmed by histology. M2-PK measurements were done by enzyme-linked immunosorbent assay of fecal occult blood test (FOBT) and immunological FOBT (IFOBT) according to the manufacturer's instructions. **Results:** M2-PK > 9 (U/mL) was the best cutoff point in the detection of colon cancers. In this cutoff point, sensitivity and specificity were 87.8% and 91.8%, respectively, and accuracy was 89.8%. The sensitivity and specificity of IFOBT were 93.9% and 100%, respectively, and accuracy was 96.9%. The sensitivity and specificity of FOBT were 65.3% and 100%, respectively, and accuracy was 82.6%. **Conclusion:** IFOBT with high sensitivity and specificity and accuracy and low cost is the best fecal screening test. The current study suggests that fecal M2-PK can be used for high-risk colon cancer patients and negative IFOBT that refused colonoscopy as a precolonoscopy screening test.

Keywords: *Colonic neoplasms, clinical laboratory techniques, colonoscopy, M2-type pyruvate kinase, occult blood*

Introduction

Colon cancer is the third most common cancer in men and the second most common cancer in women.[1] Early detection of cancer is important for cancer survival; as a result, screening programs have been set up in a variety of countries around the world. While colonoscopy has been used as a screening tool in some countries, it is invasive, has inherent (although low) risks of perforation, and the uptake by the population is low.[2] For an effective screening program, a rapid and cost-effective method is required.[3] Fecal occult blood test (FOBT)-based guaiac test is extensively used as the most frequent screening method and despite but it has low sensitivity, it is beneficial for screening. Although the newer immunological FOBT (IFOBT) method has been shown to be more sensitive, nonbleeding tumors are not diagnosed with this method.[4]

The metabolism of cancer cells is markedly different from normal cells. Cancer cells harvest large amounts of glucose and use it in a different way than normal cells. This phenomenon is known as the Warburg effect or aerobic glycolysis, which is associated with the production of lactate even in the presence of oxygen and allows tumor cells to grow in different locations with different oxygen concentrations.[5]

Cancer cells are glycolytic at high speeds to provide energy for their anabolism, which is accompanied by the expression of the pyruvate kinase isoenzyme type M2 (M2-PK). M2-PK catabolizes the last step of glycolysis and reprograms the glycolytic pathway to meet the metabolic needs of proliferative cells. Available evidence suggests an essential role for M2-PK in tumor progression.[6] Fecal M2-PK measurement has been described as a novel method for the diagnosis of colorectal cancer (CRC).[7] It has been found that dimeric M2-PK is available in the plasma of cancer patients and its concentration is related to the stage of the disease. In addition, M2-PK can be

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identified in stool of lower gastrointestinal cancer patients and it is used for colon cancer screening. In colon cancers, high M2-PK serum levels are associated with poor response to 5-Fu in chemotherapy. Its plasma level drops after complete recovery and increases with regional recurrence. In addition, M2-PK released from the tumor may stimulate angiogenesis by binding to tumor endothelial marker 8.[6] Similar to FOBT, this method is able to detect fecal tumor M2-PK using preformed cassettes. However, the effectiveness of this method for detecting colon cancers and colonic adenoma has not yet been established. A new qualitative M2-PK sample has recently been introduced for clinical use.[8] Given that so far there is no comparison between FOBT and IFOBT and fecal M2-PK testing.[9] The purpose of the study was to determine the diagnostic accuracy of fecal M2-PK and FOBT and IFOBT in comparison with colonoscopy for colon cancers and to specify its use in colon cancer screening.

Materials and Methods

The present study is a case–control and was performed with the approval of the Institutional Review Board of Isfahan University of Medical Sciences. Between March 2018 and March 2019, 49 patients with colon cancers and 49 healthy controls were selected consecutively among individuals undergoing screening colonoscopy for various indications at colonoscopy clinic at Al-Zahra Hospital, Isfahan, Iran, and private office. The diagnosis of colon cancers was based on colon cancers histologically confirmed on colonoscopy and biopsy. The cases and controls were selected among patients undergoing colon cancer screening or patients who presented with signs of tenesmus, alteration in bowel habit, bleeding, weight loss, abdominal pain, abdominal distention, and unexplained iron deficiency anemia. Patients with evidence of pancreatic, gastric, esophageal, and cholangiocellular cancer and those with inflammatory bowel disease were excluded from the study, due to increased M2-PK in this disorder.[10] The study participants were informed about the study details and written informed consent was obtained from them.

Collected data were included the age, sex, addiction and alcohol status, family history of colon cancers, colonoscopy reasons (including; bleeding, alteration in bowel habit, weight loss and other reasons), location of tumor (as proximal; cecum, ascending colon, hepatic flexure, transverse colon and splenic flexure, and distal; descending colon, sigmoid colon, rectum and anal canal), presence of inflammatory bowel disease, M2-PK, FOBT and IFOBT.

The stool samples, for the assessment of M2-PK, FOBT, and IFOBT, were collected, at least 1 week after the colonoscopy. Samples were tested as soon as collected; otherwise, samples were kept at 4° centigrade for maximum of 24 h. All samples were analyzed at a single central laboratory. The M2-PK was measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. FOBT and IFOBT were measured using commercially available immunochemical kits according to the manufacturer's instructions.

The statistical analysis was performed using MedCalc for Windows version 10.2 (MedCalc software, Mariakerke, Belgium). Findings reported as mean ± standard deviation or number (%) as appropriate. Independent sample *t*-test and Chi-square test were used to compare variables between cases and controls when appropriate. A receiver operating characteristic (ROC) curve analysis was used to evaluate the areas under the ROC curve which established the best cutoff values for M2-PK to diagnose CRC. Estimates with 95% confidence intervals (CI) of sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs), and positive and negative likelihood ratio were calculated for M2-PK, FOBT, and IFOBT to diagnose colon cancers. The results were considered statistically significant when $P < 0.05$.

Results

Table 1 shows the demographic characteristics of case and control groups. The mean of age and sex distribution were similar ($P > 0.05$). The distribution of IFOBT, FOBT, and M2-PK is shown in Table 2.

Based on ROC analysis, M2-PK >9 (U/mL) was the best cutoff point in the detection of CRC [Figure 1]. In this cutoff point, the area under the ROC curve was 0.950 ($P = 0.0001$); sensitivity and specificity were 87.8% and 91.8%, respectively. PPV was 91.5% and NPV was 88.2% with accuracy of 89.8%. Among other cutoff points, the highest sensitivity (100%, 95% CI, 92.7–100) was obtained for M2-PK > 4.18 (U/mL) with specificity of 71.4% (95% CI, 92.7–100), and also, the highest

Table 1: Characteristics of studied groups

Variables	Cases (*n*=49)	Controls (*n*=49)	*P*
Age (year)	59.2±12.3	57.3±13.5	0.458
Sex			
Male	25 (51)	28 (57)	0.543
Female	24 (49)	21 (43)	
Addiction	6 (12.2)	4 (8.2)	0.738
Alcohol	5 (10.2)	2 (4.1)	0.436
Family history	6 (12.2)	5 (10.2)	0.737
Colonoscopy reasons			
Bleeding	27 (55.1)	17 (34.7)	0.042
Alteration in bowel habit	19 (38.8)	24 (49)	0.309
Weight loss	17 (34.7)	10 (20.4)	0.113
Screening without sign	2 (4.1)	6 (12.2)	0.268
Location of tumor			
Proximal	23 (47)	-	
Distal	26 (53)	-	

Data are mean±SD or *n* (%). *P* values calculated by independent sample *t*-test or Chi-square test. SD: Standard deviation

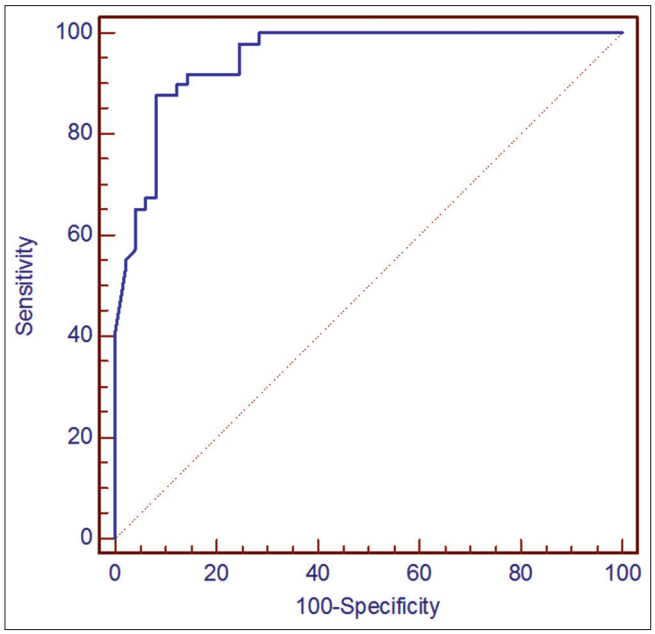

Figure 1: The receiver operating characteristics curve of M2-PK for the diagnosis of colorectal cancer. The curve is statistically significant (*P* = 0.0001). The best cutoff point was M2-PK > 9 (U/mL) with area under the receiver operating characteristics curve 0.950, 95% confidence interval 0.887–0.984

specificity (100%, 95% CI, 92.7–100) was obtained for M2-PK > 20 (U/mL) with sensitivity of 40.8% (95% CI, 27.0–55.8) [Table 3].

Discussion

Today, colonoscopy seems to be the most sensitive and specific method for CRC screening. However, the acceptance of this screening method by the general population is very poor. Therefore, we are in urgent need for other screening strategies.[11]

The current study was done with the aim of comparing the three methods of IFOBT, FOBT, and M2-PK™ Stool Test with colonoscopy as the gold standard of colon cancer diagnosis. The results showed that IFOBT was negative in 6% of the cases and about FOBT, it was negative in 35%, whereas in the control group, all the participants had negative IFOBT and FOBT. It has shown that both of the IFOBT and FOBT had the specificity of 100% in detecting colon cancers. In our study, there was no relationship between age, sex, alcohol consumption, addiction, and family history; bleeding was the most frequent reason for colonoscopy in the cases, whereas in the controls, alteration in bowel habit was the main reason. Tumors in half of the studied patients were proximal.

M2-PK, commonly upregulated in numerous cancers, plays a critical role in glucose metabolism, as well as being essential in gene transcription and tumorigenesis.[12]

In a study, Rong Cui was observed that the M2-PK mRNA level was generally upregulated in colon cancer tissue compared to the normal type. Extensive immunohistochemical analyzes in 345 colon cancer samples have shown that the incidence of M2-PK is positive in 79% and is associated with enhanced tumor, node metastasis stage, and higher carcinoembryonic antigen (CEA) levels. Higher M2-PK is associated with worse clinical outcomes. M2-PK can serve as a molecular target for colon cancer treatment.[9]

In a study conducted by Li *et al*., the diagnostic value of M2-PK in the stool for CRC screening was evaluated by systematic review and meta-analysis. However, the sensitivity and specificity of this test have been expressed differently in different studies. The purpose of their study was to determine the diagnostic accuracy of fecal M2-PK in comparison with the guaiac FOBT and IFOBT for colon cancers and to specify its use in colon cancer screening. In summary, 79% sensitivity, 81% specificity, 79% PPV, and 86% NPV were obtained. They concluded that fecal M2-PK as a diagnostic test has moderate sensitivity and

Table 2: Comparison of immunological fecal occult blood test, fecal occult blood test, and M2-PK between studied participants

test	Cases (%)	Controls (%)	*P*
IFOBT			
Positive	46 (94)	0	0.0001
FOBT			
Positive	32 (65)	0	0.0001
M2-PK by cutoff>9			
Positive	43 (88)	4 (8)	0.0001
M2-PK, (U/mL)	20.6±8.4	4.3±4.9	0.0001

Data are mean±SD or *n* (%). *P* values calculated by independent sample *t*-test or Chi-square test. SD: Standard deviation

Table 3: Diagnostic values of immunological fecal occult blood test, fecal occult blood test, and M2-PK for detecting colorectal cancer

Diagnostic values	Estimates (95% CI)		
	IFOBT	FOBT	M2-PK>9 (U/mL)
Sensitivity	93.9 (83.1–98.7)	65.3 (50.4–78.3)	87.8 (75.2–95.3)
Specificity	100 (92.7–100)	100 (92.7–100)	91.8 (80.4–97.7)
Positive likelihood ratio	-	-	10.7 (9.4–12.3)
Negative likelihood ratio	0.06 (0.02–0.18)	0.35 (0.24–0.51)	0.13 (0.04–0.4)
Positive predictive value	100	100	91.5 (79.6–97.6)
Negative predictive value	94.2 (84.5–98.0)	74.2 (66.5–80.9)	88.2 (76.1–95.5)
Accuracy	96.9 (91.31–99.0)	82.6 (73.7–89.6)	89.8 (82.0–95.0)

IFOBT: Immunological fecal occult blood test, FOBT: Fecal occult blood test, CI: Confidence interval

specificity for the determination of colon cancers, and its diagnostic efficiency is as much as gFOBT. Because of the low sensitivity and low PPV, therefore fecal M2-PK alone is not suggested as a screen for colon cancers.[7] This study states that neither gFOBT nor IFOBT is specific for colon cancers, as any intracellular hemorrhage can give a positive result to the test. For this reason, as a marker tumor that is released from the tumor tissue, M2-PK is superior for the diagnosis of bleeding or nonbleeding neoplasms. However, the combination of fecal M2-PK and IFOBT may diagnose more patients with than each of them alone.[7]

A study by Suresh Sithambaram *et al*. showed that fecal testing, which is widely used for colorectal screening, is based on finding occult blood in the stool. The guaiac test has been used for a long time, but it has high false-positive results, which is due to the nature of the test based on its oxidative ability, it has a sensitivity of 79.9%, and specificity of 86.7%. IFOBT is based on the measurement of globulin, and the globulin that originates from the upper gastrointestinal tract rapidly dissolves in the gastrointestinal tract. As a result, this test is highly selective for occult blood of colorectal origin with 70%–90% sensitivity for CRC diagnosis. In the colon cancer, M2-PK is secreted in the colon lumen. The sensitivity, specificity, PPV, NPV, and overall accuracy were 93%, 97.5%, 94.9%, 96.5%, and 96%, respectively.[8]

A study by Ahmet Sutdemir has shown that M2-PK levels may be useful in the diagnosis of malignant colon versus benign lesions. In this study, 85 patients with neoplastic lesions diagnosed by colonoscopy were enrolled. The patients were divided into two groups based on macroscopic evidence of polyp or carcinoma. On the basis of histopathologic lesions, the specimens were classified into nonneoplastic, tubular adenomas, and adenocarcinoma groups. M2-PK was measured with an ELISA kit, and its plasma level was determined to be 76.1 M2-PK.

The carcinoma group had the highest levels of M2-PK both endoscopically and histopathologically. M2-PK levels of patients who died were significantly higher than patients who survived.[12]

A study by Philippe de Hardt has shown that fecal tumor M2-PK testing resembles a good noninvasive screening parameter for CRC with a reported sensitivity of 68.8%–91.0% and a specificity of 71.9%–100%. It is superior to fecal occult blood testing in CRC screening. Since it is effective, easy to handle, and bears rather low costs, fecal tumor M2-PK testing is recommended for large-scale CRC screening.[11] In this article, it is stated that no comparison has been made between FOBT and the fecal test.

In the study by Yu Hong, it has been indicated that fecal testing for a marker tumor may be a noninvasive colorectal screening method. The aim of this study was to evaluate the potential of a new fecal M2-PK tumor marker test to isolate colon cancer patients from elderly patients in a large nonselective sampling. The fecal M2-PK of 65 patients with colon cancer and 917 individuals from the population with mean age of 65 years and 62 years were determined. The mean value of M2-PK was 8.6 uml in patients with colon cancer and in the study population was <2 uml. At the 4 uml cross-sectional area 85%, sensitivity was 56% for colon cancer and specificity was 56% for rectal cancer. Given the high sensitivity of M2-PK stool test, especially for colon cancer and the ease of screening and the potential of using this test for early colon cancer screening; it is necessary to do further investigation.[10]

According to the results of our study, the mean value of M2-PK was significantly higher in the patients than the control group, and M2-PK > 9 (U/mL) was the best cutoff point in the detection of colon cancer. In this cutoff point, the sensitivity and specificity were 87.8% and 91.8% respectively. PPV was 91.5% and NPV was 88.2% with accuracy of 89.8%; the highest sensitivity (100%, 95% CI, 92.7–100) was obtained for M2-PK > 4.18 (U/mL) with specificity of 71.4% (95% CI, 92.7–100), and also, the highest specificity (100%, 95% CI, 92.7–100) was obtained for M2-PK > 20 (U/mL) with sensitivity of 40.8% (95% CI, 27.0–55.8).

IFOBT with high sensitivity and specificity and accuracy and low cost is the best fecal screening test. The current study suggests that fecal M2-PK can be used for high-risk colon cancer patients and negative IFOBT that refused colonoscopy as a precolonoscopy screening test. It is a feasible tool to preselect patients who require colonoscopy. This study suggests that further studies are needed to increase the specificity of M2-PK, by combining with IFOBT.

Study limitations

Our study had limitations such as small sample size and impossibility to increase sample size due to financial and time constraints. Therefore, further studies are recommended.

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Conflicts of interest

There are no conflicts of interest.

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