EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 5
139-143
Year: 2016

M2 PYRUVATE KINASE IN COLORECTAL CANCER IN ANEMIC PATIENTS

Mohamed Metwally[a], Mohammed Mohammed Shamseya[b], Ahmed E. Alakany[c], Mohamed M. El Sawy[d], El-Moughazi AM.[e]*

[A]Consultant of Tropical Medicine in Alexandria Fever Hospital – Embrouzo, Elhadara Infront of Nariman Hospital, Alexandria, Egypt.
[B]Assistant Professor in Department of Clinical and Experimental Internal Medicine; Medical Research Institute, Alexandria University - 165 Abou Qir St., Elhadara Elbahreya, Alexandria, Egypt.
[C]Lecturer, Department of Internal Medicine; Faculty of Medicine, Alexandria University - Elsultan Hussein St., Elqhartoum Square, Aleandria, Egypt.
[D]Assistant Professor, Department of Clinical Pathology; Faculty of Medicine, Alexandria University – Elsultan Hussein St., Elqhartoum Square, Alexandria, Egypt.
[E]Resident in Department of Clinical and Experimental Internal Medicine; Medical Research Institute, Alexandria University - 165 Abou Qir St., Elhadara Elbahreya, Alexandria, Egypt.

*** Corresponding Author: Dr. Ahmed Elmoughazy**
Resident in Department of Clinical And Experimental Internal Medicine; Medical Research Institute, Alexandria University - 165 Abou Qir St., Elhadara Elbahreya, Alexandria, Egypt.,

Article Received on 14/03/2016 **Article Revised on 03/04/2016** **Article Accepted on 24/04/2016**

ABSTRACT
Background: Colorectal cancer (CRC) is a disease with a major impact on public health and public health costs. Colonoscopy is the gold standard screening tool for CRC. However, its acceptance by the general population is relatively low; hence evaluation of other non-invasive screening tools is essential. M2 pyruvate kinase (M2PK) appears to be a good candidate, yet its accuracy is not yet fully assessed. **Aims:** To Estimate sensitivity, specificity, positive (PPV) and negative (NPV) predictive value of M2PK relative to colonoscopy in screening of CRC in anemic patients with unexplained bleeding per rectum and unintentional weight loss in Egypt. **Methods:** Thirty consecutive cases of anemia complaining of unexplained bleeding per rectum and unintentional weight loss were subjected after taking informed consent to colonoscopy and multiple biopsies were taken for histopathological assessment. Fecal tumor M2PK level was measured using an ELISA kit in both groups. **Results:** Sensitivity was (75%), specificity (60%), PPV (78%) and NPV (54.5%). **Conclusion:** Measurement of tumor M2PK in stool seems to be a promising tool for CRC screening at present time in combination with colonoscopy.

KEYWORDS: Colorectal cancer, Colonoscopy, M2PK, Egypt, Screening.

INTRODUCTION
Every year approximately one million people worldwide are diagnosed with colorectal cancer and nearly 529,000 people die from this neoplasm.[1] Due to slow development of the disease, colorectal cancer screening is particularly effective. Therefore, regular screening examinations can significantly reduce cancer morbidity and mortality. Several guidelines for colorectal cancer screening in different countries have been proposed, such as the fecal occult blood test, sigmoidoscopy, double-contrast barium enema, or colonoscopy.[2-5]

Although colonoscopy is the gold standard for the early detection of colorectal cancer and its precancerous lesions, it has some drawbacks. The procedure is invasive, expensive, associated with the highest rates of complications[6] and has low acceptance by the general population. For example, only 1.7% of those people entitled to colonoscopy under the German National Colorectal Cancer Screening Program actually undergo the procedure.[7] In contrast to colonoscopy, the fecal occult blood tests (FOBTs) are widely accepted.[3-5] They are based on the premise that polyps and cancers bleed more than normal mucosa.[8]

Consequently, non-bleeding colo-rectal tumors or polyps and those not consistently discharging sufficient blood into the intestinal lumen are not detected by either guaiac or immunological FOBTs. Therefore, the sensitivities of these tests are limited. For the guaiac- based FOBT, sensitivities of less than 30% for colorectal cancer and less than 15% for advanced adenomas have been described by Lieberman et al[9] and by Koss et al.[10] In addition, there is a need for both dietary and medication restrictions because certain foods (such as red meat, and some raw foods and vegetables), vitamin C or aspirin may lead to false-positive results. This has a negative impact on the specificity of guaiac-based

FOBTs.[11] However, despite these limitations, guaiac-based FOBTs have been shown to reduce mortality in the range of 15-33% in screened populations.[12, 13]

Even though the disadvantage of dietary restriction has been overcome by the development of more expensive immunological FOBTs, the problems of detecting intermittently bleeding cancers and precancerous polyps is still a problem.

In order to increase participation in colorectal cancer screening programs, a non-invasive, easy, fast, and economical screening method is needed. Further it should not be dependent upon the presence of occult blood and should have good patient compliance, high sensitivity and high specificity. Here in this study we are targeting a special subgroup of the patients who has frank bleeding per rectum without obvious cause, which make FOBTs even not practical and of no use.

The M2PK stool test is therefore of interest, as it is a screening test for the detection of adenomas and colorectal tumors. It is based on the measurement of a key enzyme involved in tumor metabolism[10, 14-21] and is independent of the presence of occult blood. Tumor M2PK is the dimeric form of the glycolytic pyruvate kinase isoenzyme type M2.[22, 23] This enzyme catalyzes the last reaction step within the glycolytic sequence, from phosphor-enolpyruvate to lactate, and is responsible for net ATP production within this pathway. Enzymatic characterization of a wide range of different tumors revealed that oncogenesis is accompanied by an increase in total pyruvate kinase v-max activity. Fecal M2PK is stable for 48 hours at room temperature, 72 hours at 4–8°C, and 1 year at –2°C. Undiluted stool extracts may be stored without adverse effects for 1 day at 4–8°C or for 4 weeks at –20°C.[24]

PATIENTS AND METHODS

Thirty patients were included complaining of significant weight loss (more than 10% of body weight), anemia (defines as hemoglobin less than 14 gm/dl for males and less than 12.3 gm/dl for females[25]), bleeding per rectum. Patients were subjected - After informed consent according to Declaration of Helsinki- to colonoscopy (Figure. 1) and biopsy taking for histopathological assessment. Patients with any other disease causing upper and lower GIT bleeding like liver cirrhosis, cancer stomach, angiodysplasia, diverticulosis, hemorrhoids &/or fissures were excluded.

All individuals were subjected to Stool sampling; one stool sample was collected before bowel preparation for colonoscopy or after colonoscopy when the patient's stool had become sufficiently firm. A clean plastic container (100 mL) and a plastic spoon were provided for stool collection. No dietary restrictions were imposed. The stool sample collection procedure was explained to the patient by nursing staff after being trained to explain the standard collection method. Patients who returned very watery stool samples were asked to collect a firm stool sample.

Patients were instructed not to contaminate stool samples with toilet bowl water. Stool extraction was done using the FECAL TUMOR M2PK QUICK PREP KIT (SCHEBO BIOTECH AG, GIESSEN, GERMANY). Fecal tumor M2PK level was measured using an ELISA kit (SCHEBO BIOTECH AG) in accordance with the manufacturer's protocol. The kit was validated for quantification of fecal tumor M2PK at any level. An M2PK cutoff level of 4 U/mL was used according to the manufacturer's instructions and other similar studies. Patients whose fecal tumor M2PK levels were greater than the cutoff level were classified as positive for tumor M2PK.[24]

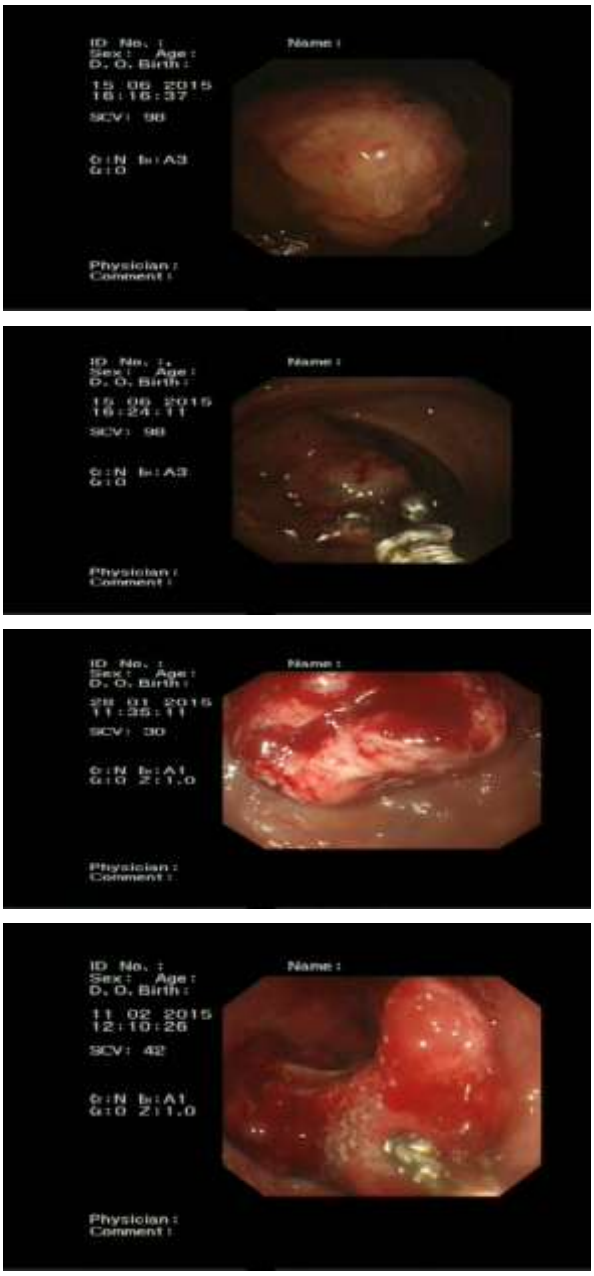

Figure (1): Colonoscopy showing cancer colon.

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 20) software. Categorized data was presented as number and percent while the

numerical data was presented as mean, standard deviation, for comparison between the two groups t-test was used. The level of significance was 0.05. ROC curve (Receiver operating characteristic curve) was done to determine the cut off value of M2PK marker in detection of positive cases.

RESULTS

Table (1) shows the characteristic features (sex, age) of the studied groups. Males were 27 (90%) and females were 3 (10%). Age < 50 was 11 (36.7%) and >50 was 19(63.3%), it ranged between 40-62 years with a mean value 54.6±6.25. Mean of Hemoglobin level among the selected patients was 9.1 gm/dl. Table (2) shows sensitivity, specificity, positive and negative predictive values of M2PK marker in relation to colonoscopy as a gold standard method. Sensitivity was (75%), specificity (60%), PPV (78.9%) and NPV (54.5%).

Table (3) shows comparison between the patients positive and negative by colonoscopy and the level of M2PK. M2PK in positive colonoscopy ranged 2.11-30.1 U/mL with mean value 16.07±8.82 and negative colonoscopy ranged 0.67-22.6 U/mL with mean value 8.48±9.41.

Table (1): Characteristic features of the studied groups.

	Number	Percent
Sex		
Male	27	90.0
Female	3	10.0
Age (years)		
<50	11	36.7
>50	19	63.3

Table (2): Sensitivity, specificity, positive and negative predictive values of M2 PYRUVATE KINASE marker in relation to colonoscopy.

M2-PK	Colonoscopy				Total
	Positive		Negative		
	No.	%	No.	%	
Positive	15	75.0	4	40.0	19
Negative	5	25.0	6	60.0	11
Total	20	100.0	10	100.0	30
Sensitivity	75.0%				
Specificity	60.0%				
PPV	78.9%				
NPV	54.5%				

Table (3): Comparison between the patients positive and negative by colonoscopy and the level of M2 PYRUVATE KINASE.

M2-PK	Colonoscopy	
	Positive	Negative
Range	2.11-30.1	0.67-22.6
Mean	16.07	8.48
S.D.	8.82	9.41
T P	**3.25 0.001***	

There was statistical significant difference between the patients positive and negative by colonoscopy and the level of M2PK (P < 0.05). (Figure. 2).

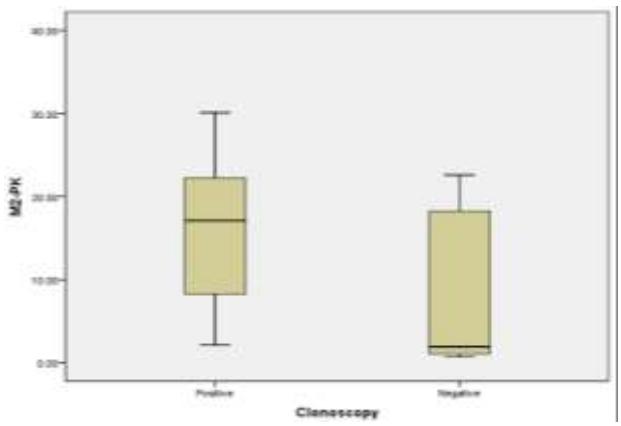

Figure (2): Comparison between the patients positive and negative by colonoscopy and the level of M2PK.

The ROC curve (Figure. 3) was done to determine the cut off value of the M2PK in detection of patients with positive disease, this cut off value was 4.595 U/mL, the sensitivity of this point was 75.0% while the specificity was 60.0%.

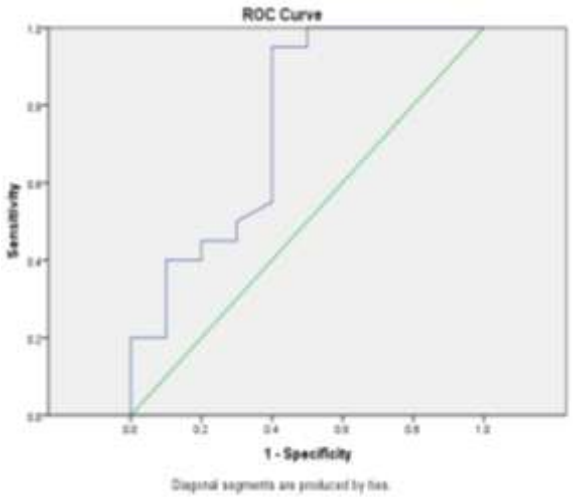

Figure (3): ROC curve to determine the cut off value of M2PK to determine the sensitivity and specificity.

Area Under the Curve				
Test Result Variable(s):M2PK				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.753	0.105	0.026	0.547	0.958

Coordinates of the Curve		
Test Result Variable(s):M2PK		
Cut off value	Sensitivity	Specificity
4.5950	0.750	0.600

DISCUSSION

This is to the best of our knowledge the first study to assess the potential use of M2PK as a screening tool for colorectal cancer in the Middle East. Previous studies describe that tumor M2PK is released into the stool of patients with adenomas and colorectal tumors and can easily be quantified with a commercially available sandwich ELISA.[16, 26, 27]

In our study 10 patients were negative for colorectal cancer by colonoscopy but 4 of them were positive for M2PK test. Those 4 patients were found to have ulcerative colitis. Naumann et al[28] found increased fecal tumor M2PK levels in cases of active Crohn's disease and ulcerative colitis in which increased cell proliferation is expected. Sensitivity was (75%), specificity (60%), PPV (78.9%) of M2PK marker in relation to colonoscopy as a gold standard.

Although a larger similar study has reported sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of: 93%, 97.5%, 94.9%, 96.5% and 96.0% respectively[29] and a higher sensitivity was reported by McLoughlin et al[17] (92% in 25 colorectal cancer patients and 67% in 30 patients with adenomas) and by Koss et al[10] (92.3 % in 26 colorectal cancer patients and 60% for adenomas >1 cm in 10 patients), our data corresponds well with the results of Hardt et al[16] who reported a sensitivity of 78% in 60 colorectal cancer patients and those of Naumann et al[28] who found a sensitivity of 85.2% in a cohort of 27 colorectal cancer patients as well as a recent systematic meta-analysis of 8 trials showing a pooled sensitivity and specificity for M2-PK to be 79% (CI 73%-83%) and 80% (CI 73%-86%), respectively.[30]

CONCLUSION

M2PK test had a high sensitivity and specificity for colorectal cancer in anemic patients with bleeding per rectum without obvious cause. However we believe that further larger trials are needed for accurate determination of diagnostic cut-off level.

REFERENCES

1. Parkin DM, Bray F, Ferlay J et al. Global cancer statistics 2002. CA Cancer J Clin, 2005; 55: 74-108.
2. Smith RA, Cokkinides V, Eyre HJ. Cancer screening in the United States, 2007: a review of current guidelines, practices and prospects. CA Cancer J Clin., 2007; 57: 90-104.
3. Haug U, Rothenbacher D, Wente et al. Tumor M2-PK as a stool marker for colorectal cancer: comparative analysis in a large sample of unselected older adults vs colorectal cancer patients. Br J Cancer., 2007; 96: 1329-34.
4. Rhodes JM. Colorectal cancer screening in the UK: Joint Position Statement by the British Society of Gastroenterology, The Royal College of Physicians & The Association of Coloproctology of Great Britain and Ireland. Gut., 2000; 46: 746-8.
5. Australian Cancer Network Colorectal Cancer Guidelines Revision Committee. Guidelines for the Prevention, Early Detection and Management of Colorectal Cancer. Sydney: The Cancer Council Australia & Australian Cancer Network, 2005.
6. Waye JD, Kahn O, Auerbach ME. Complications of colonoscopy and flexible sigmoidoscopy. Gastrointest Endosc Clin N Am, 1996; 6: 343-7.
7. Shastri YM, Naumann M, Oremek GM et al. Prospective multicenter evaluation of fecal tumor pyruvate kinase type M2 (M2-PK) as a screening biomarker for colorectal neoplasia. Im J Cancer, 2006; 119: 2651-6.
8. Macrae FA, St.John DJB. Relationship between patterns of bleeding and hemoccult sensitivity in patients with colorectal cancers or adenomas. Gastroenterology., 1982; 82: 891-8.
9. Lieberman DA, Harford WV, Ahnen DJ et al. One-time screening for colorectal cancer with combined fecal occult-blood testing and examination of the distal colon. N Engl J Med., 2001; 345: 555-60.
10. Koss K, Maxton D, Jankowski JA. Faecal dimeric M2 pyruvate kinase in colorectal cancer and polyps correlates with tumour staging and surgical intervention. Colorectal Dis., 2008; 10: 244-8.
11. Ransohoff DF, Lang CA. Screening for colorectal cancer with the fecal occult blood test: a background paper. American College of Physicians. Ann Intern Med., 1997; 126: 811-22.
12. Hardcastle JD, Chamberlain JO, Robinson MHE et al. Randomised controlled trial of faecal occult blood screening for colorectal cancer. Lancet., 1996; 348: 1472-7.
13. Mandel J, Church TR, Ederer F, et al. Effectiveness of biennial screening for fecal occult blood. J Natl Cancer Inst., 1999; 91: 434-7.

14. Ewald N, Toepler M, Akinci A et al. Pyruvatkinase M2 (Tumor M2-PK) Screening-parameter for colorectal cancer. Z Gastroenterol., 2005; 43: 1313-7.
15. Hardt PD, Toepler M, Ngoumou B et al. Measurement of fecal pyruvate kinase type M2 (Tumor M2-PK) concentrations in patients with gastric cancer, colorectal cancer, colorectal adenomas and controls. Anticancer Res., 2003; 23(2A): 851-3.
16. Hardt PD, Mazurek S, Toepler M et al. Faecal tumour M2 pyruvate kinase: a new, sensitive screening tool for colorectal cancer. Br J Cancer., 2004; 91: 980-4.
17. McLoughlin R, Shiel E, Sebastian S et al. Tumor M2-PK, a novel screening tool for colorectal cancer. In: Poster Abstracts & Trade Exhibition Book: NCRI Cancer Conference; 2005 Oct 2-5; Birmingham, United Kingdom. London: Callisto, 2005; 202.
18. Tonus C, Neupert G, Sellinger M. Colorectal cancer screening by noninvasive metabolic biomarker fecal tumor M2-PK. World J Gastroenterol., 2006; 12: 7007-11.
19. Ewald N, Schaller M, Bayer M et al. Fecal pyruvate kinase-M2 (tumor M2-PK) measurement: a new screening concept for colorectal cancer. Anticancer Res., 2007; 27: 1949-52.
20. Kumar Y, Tapuria N, Kirmani N et al. Tumour M2-pyruvate kinase: a gastrointestinal cancer marker. Eur J Gastroenterol Hepatol., 2007; 19: 265-76.
21. Hathurusinghe HR, Goonetilleke KS, Siriwardena AK. Current status of tumor M2 pyruvate kinase (tumor M2-PK) as a biomarker of gastrointestinal malignancy. Ann Surg Oncol., 2007; 14: 2714-20.
22. Eigenbrodt E, Reinacher M, Scheefers-Borchel U et al. Double role for pyruvate kinase type M2 in the expansion of phosphor-metabolite pools found in tumor cells. Crit Rev Oncogenesis., 1992; 3: 91-115.
23. Christofk HR, Van der Heiden MG, Wu N et al. Pyruvate kinase M2 is a phosphotyrosine-binding protein. Nature., 2008; 452: 181-6.
24. Peeters M, Haller DG. Therapy for early stage colorectal cancer. Oncol., 1999; 13: 307–15.
25. 1. Hemoglobin Concentration (Hb): Reference Range, Interpretation, Collection and Panels. 2015 Jun 5 [cited 2015 Oct 9]; Available from: http://emedicine.medscape.com/article/2085614-overview.
26. Koss K, Maxton D, Jankowski JAZ. The potential use of fecal dimeric M2 pyruvate kinase (Tumour M2-PK) in screening for colorectal cancer (CRC). In: Poster Abstracts and Trade Exhibition Book: NCRI Cancer Conference; 2005; 2-5. Birmingham, UK. London: Callisto, 2005; 199.
27. Ewald N, Toepler M, Akinci A, et al. Pyruvate kinase M2 (tumor M2-PK) as a screening tool for colorectal cancer (CRC). A review of current published data. Z Gastroenterol, 2005; 43: 1313-7.
28. Naumann M, Schaum B, Oremek GM, et al. Faecal pyruvate kinase type M2--a valid screening parameter for colorectal cancer? Preliminary results from a multicenter comparative study. Dtsch Med Wochenschr., 2004; 129: 1806-7.
29. Sithambaram S, Hilmi I, Goh K-L. The Diagnostic Accuracy of the M2 Pyruvate Kinase Quick Stool Test- A Rapid Office Based Assay Test for the Detection of Colorectal Cancer. PLoS ONE., 2015; 10(7): e0131616.
30. Uppara M, Adaba F, Askari A, Clark S, Hanna G, Athanasiou T, et al. A systematic review and meta-analysis of the diagnostic accuracy of pyruvate kinase M2 isoenzymatic assay in diagnosing colorectal cancer. World J Surg Oncol., 2015; 13: 48.