RESEARCH ARTICLE

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Validation of Faecal Pyruvate Kinase Isoenzyme Type M2 (Faecal M2PK Quick) Test in Detection of Colorectal Adenoma and Adenocarcinoma Among High-Risk Malaysian Population

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Abstract

Background: Colorectal neoplasia is a multistep process that can lead to the development of colorectal cancer. Colonoscopy is the gold standard for diagnosis and screening of colorectal cancer, but its uptake is often hindered by unpleasant experiences and logistic obstacles. Therefore, non-invasive biomarker tests such as the M2-pyruvate kinase (M2PK) test have been explored as a potential screening tool. **Objective:** This study aims to evaluate the efficacy of the M2PK Quick Stool Test (ScheBo®) in detecting colorectal adenoma and adenocarcinoma in high-risk Malaysian populations using colonoscopy as the comparison. **Methods:** A prospective, cross-sectional, multicenter study was conducted from December 2017 to December 2019 in four hospitals in Malaysia. Participants were eligible if they met any of the following criteria: personal or family history of colorectal polyps or cancer, inherited syndromes, altered bowel habits, rectal bleeding, unintended weight loss, loss of appetite, abdominal pain or cramps, or unexplained iron deficiency, or an Asia-Pacific Colorectal Screening score of 4-7. Participants provided a stool sample that was tested for M2PK using the M2PK Quick Test. Participants then underwent a colonoscopy, and any lesions found were biopsied and sent for histopathological examination. **Results:** A total of 562 participants were included in the study, of whom 89 had a positive M2PK test. Presence of adenoma and/or dysplastic lesions were confirmed in 14.4% and adenocarcinoma in 3.0% of the participants. The M2PK Quick Stool Test showed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 58.8%, 85.5%, 11.2% and 98.5%, respectively in detecting colorectal adenocarcinoma. For detection of colorectal adenoma, this test yielded a sensitivity, specificity, PPV and NPV of 27.3%, 86.3%, 27.0% and 86.5%, respectively. **Conclusions:** The M2PK Quick Stool Test showed a moderate accuracy in detecting colorectal adenocarcinoma and adenomas in the studied population.

Keywords: Diagnostic accuracy- colorectal neoplasm- M2-Pyruvate kinase- tumour biomarkers

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Introduction

The development of colorectal neoplasia is a multistep process involving the accumulation of genetic and epigenetic alterations in the cells lining the colon and rectum (Manne et al., 2010; Schlussel et al., 2014). It typically begins as the formation of benign growths known as polyps, which can be either adenomatous or non-adenomatous. Adenomatous polyps, also known as adenomas, and other colon dysplastic lesions are considered precancerous lesions, as they have the potential to progress into colorectal cancer over time (Neugut et al., 1993). If left untreated, adenomas could undergo further genetic mutations and changes in cell behaviours, leading to the development of invasive adenocarcinoma. However, this process can take several years, providing an opportunity for early detection and intervention through regular screening to detect colorectal cancer at an early and treatable stage.

Clinical guidelines generally recommend colonoscopy as the gold standard for the diagnosis and screening of colorectal cancer and adenomas (Sung et al., 2022). However, colonoscopy uptake is often hindered by unpleasant experiences, fear of the procedure, and logistic

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obstacles (Mohd Suan et al., 2020). Therefore, utilizing non-invasive biomarkers as a complementary screening tool is necessary.

The immunological Faecal Occult Blood Test (iFOBT) is a widely used stool-based biomarker test that detects human haemoglobin using globin-specific antibodies (Allison et al., 2014). In Malaysia, the Ministry of Health Malaysia launched a nationwide colorectal cancer screening programme using the iFOBT in 2014 to promote early diagnosis of colorectal cancer. The target group of this programme is individuals between 50 and 75 years of age who are at average risk of developing colorectal cancer. Despite the benefit of the iFOBT, its uptake has also been suboptimal, mainly due to fear of the test outcome, hygiene issues related to stool collection and a lack of recommendations from healthcare providers (Bujang et al., 2021).

Another potential stool-based biomarker test is the M2-pyruvate kinase (M2PK) test. M2PK is an isoenzyme of pyruvate kinase that is released into the colon by the tumour and can be detected in stool samples (Mazurek et al., 2005). Although The M2PK test has been examined in numerous trials for colorectal cancer screening, there have been conflicting findings regarding its sensitivity, specificity and overall accuracy (Nasir Kansestani et al., 2022; Uppara et al., 2015). Therefore, the purpose of this study was to evaluate the efficacy of the M2PK quick stool test (ScheBo®) in detecting colorectal cancer and adenoma in high-risk Malaysian populations using colonoscopy as the comparison.

Materials and Methods

Study design and procedures

This prospective, cross-sectional, multicenter study was conducted from December 2017 to December 2019 in four hospitals in Malaysia. The ethics approval was granted by the Medical Research Ethics Committee (MREC) of the Ministry of Health (NMRR-17-1830-36344). Individuals who visited the Gastroenterology or Surgical Clinic at the participating hospitals were eligible for the study if they met any of the following criteria: (I) a personal or family history of colorectal polyps or cancer; (II) inherited syndromes such as familial adenomatous polyposis (FAP), hereditary non-polyposis colorectal cancer (HNPCC), or Peutz-Jegher syndrome; (III) altered bowel habits, per rectum bleeding, unintended weight loss, loss of appetite, abdominal pain or cramps, or unexplained iron deficiency; (IV) an Asia-Pacific Colorectal Screening (APCS) score of 4-7 (Yeoh et al., 2011). Individuals with a recent history (within 4 weeks) of bowel infection, inflammatory bowel disease, pregnancy, colonoscopy refusal, inability to follow stool collection instructions, and a history of colorectal cancer, were excluded.

Individuals who provided consent were instructed to collect a stool sample at home prior to the day of their colonoscopy appointment. The M2PK protein in the stool was tested at the clinic using the rapid M2PK Quick Test (ScheBo® Biotech AG, Giessen, Germany) according to the manufacturer's instructions. The interpretation was based on the appearance of bands in the test (T) and control (C) sections of the test strip after the stool sample was solubilized. Subsequently, participants underwent a colonoscopy. The endoscopist was blinded to the M2PK test result. Any lesions found during the colonoscopy were biopsied, and the tissue samples were sent to the laboratory at the respective study site for histopathological examination (HPE). The diagnosis of colorectal cancer and adenomas were confirmed based on the HPE results. Demographic and clinical data, including the HPE results, were collected using a standardized data collection form.

Statistical Analysis

The study findings were reported according to Standards for the Reporting of Diagnostic accuracy studies (STARD) guidelines. Categorical variables were presented as frequencies and percentages, and continuous variables were presented as mean and standard deviation. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the M2PK Quick Stool Test were calculated against the reference standard for diagnosis of colorectal adenomas and adenocarcinoma (HPE results). The 95% confidence intervals were presented along with these values. All the data were analyzed using the R statistical software version 3.5.2. (Team, 2014).

Results

Patient characteristics and M2PK test outcomes

Of the 600 participants, three withdrew their consent, six did not collect stool samples and 29 did not show up for colonoscopy as scheduled. The demographic characteristics and histopathological results of the 562 participants in the current study are presented in Table 1.

Table 1. Participant Characteristics

Characteristic	N	%
Age (years), mean (SD)	57.3	13.39
Gender		
Male	307	54.8
Female	255	45.2
Ethnicity		
Malay	214	38.1
Chinese	279	49.6
Indian	62	11.0
Others	7	1.3
M2PK test result		
Positive	89	15.8
Negative	473	84.2
HPE-confirmed adenoma and/or dysplastic lesion†		
Yes	88	15.7
No	474	84.3
HPE confirmed Adenocarcinoma		
Yes	17	3.0
No	545	97.0

SD, standard deviation; †, Adenoma includes all tubular adenomas, villous adenomas and tubulovillous adenomas, while dysplastic lesions encompassed low- and high-grade dysplasia.

Table 2. Diagnostic Performance of the M2PK Test to Diagnose Colorectal Adenoma and Adenocarcinoma Using Histopathological Outcomes Following Colonoscopy as a Reference Standard (n=562).

Histopathological outcomes		M2PK Test		Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
		Positive	Negative	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
HPE-confirmed adenoma and/or dysplastic lesions†	Yes	24	64	27.3	86.3	27	86.5	77
	No	65	409	(18.3-37.8)	(82.9-89.3)	(18.1-37.4)	(83.1-89.4)	(73.3-80.5)
HPE-confirmed colorectal adenocarcinoma	Yes	10	7	58.8	85.5	11.2	98.5	84.7
	No	79	466	(32.9-81.6)	(82.3-88.4)	(7.5-16.5)	(97.4-99.2)	(81.5-87.6)

HPE, histopathological examination; CI, confident interval; PPV, positive predictive value; NPV, negative predictive value; † Adenoma includes all tubular adenomas, villous adenomas and tubulovillous adenomas, while dysplastic lesions encompassed low- and high-grade dysplasia.

Most of the participants were male (54.8%), of Chinese ethnicity (49.6%) and had a mean age of 57.3 ± 13.39 years. Only 89 of them (15.8%) had a positive M2PK test. The HPE confirmed the presence of adenoma and/or dysplastic lesions in 14.4% of the participants, and adenocarcinoma in 3.0% of them. No adverse event reported following M2PK test and colonoscopy.

Diagnostic performance of M2PK test

The sensitivity, specificity, PPV, NPV and the overall accuracy of the M2PK test are summarized in Table 2. The M2PK test demonstrated a sensitivity of 58.8%, a specificity of 85.5%, a PPV of 11.2% and an NPV of 98.5% for colorectal adenocarcinoma detection. Despite a better specificity (86.3%), M2PK test showed a low sensitivity (27.3%) and NPV (86.5%) in colorectal adenoma detection. The test also showed higher accuracy (84.7%) in detecting adenocarcinoma.

Discussion

The detection of M2PK enzyme in stool samples has led to the development of new screening test for colorectal cancer and its precancerous stage. The M2PK Quick Test, for instance, is easy to perform and provides the results within a short period of time, typically in less than 20 minutes, allowing for rapid screening and decision-making by healthcare providers. The test result is also not affected by food intake; thus, no dietary restrictions are required prior to stool sample collection.

This study showed that M2PK test is able to detect small number of colorectal adenoma and adenocarcinoma cases with accurate diagnosis when using HPE result following colonoscopy as a reference standard. Specifically, M2PK test showed a test sensitivity of 58.8% and specificity of 85.5% when used to detect adenocarcinoma in this study. This result is relatively low in sensitivity compared to the findings of other studies which demonstrated a sensitivity range of 73%-97% in detecting adenocarcinoma (Sithambaram et al., 2015). Likewise, Sithambaram et al. in a case control study conducted in one of the local university hospital reported a higher sensitivity of 93% and specificity of 97.5% for M2PK test to detect adenocarcinoma (Sithambaram et al., 2015). The low sensitivity in this study could be due to the small number of patients with HPE-confirmed adenocarcinoma in the studied population (17/562), leading to wider confidence intervals and less precise estimates of sensitivity.

The sensitivity of the M2PK test for detecting adenomas and other dysplastic lesions was only 27.3%, which means that the test may miss a significant proportion of these lesions. This low sensitivity was also observed by Tonus et al., (2012) in a systematic review of the use of M2PK for colorectal adenoma detection, which ranges between 28% and 76%. In another case-control study at a local teaching hospital, the stool M2PK test showed a sensitivity and specificity of 20.0% and 54.5%, respectively, for detecting colorectal adenomas (Alhadi et al., 2020). Therefore, M2PK test has a limited role in detecting colorectal adenomas. One of the important strengths of this study was the larger size of patient enrolled for M2PK test with colonoscopy and its multicenter study design. Adoption of clear and specific inclusion criteria is another unique strength. Nevertheless, this study only focuses on the high-risk group of patients and might affect the sensitivity and specificity in hospital-based studies. Therefore, the results should be interpreted with caution when applying to an average-risk population.

In conclusion, M2PK test showed a moderate accuracy in detecting colorectal adenocarcinoma and adenomas in the studied population. This test is less effective to be used as a screening tool for mass population screening to detect these colorectal neoplasms. However, the test can still be recommended as a first-tier screening test, particularly for those individuals with symptoms or at high risk for colorectal cancer who are still uncertain or hesitate to undergo colonoscopy procedures. Only those with positive M2PK test need counselling and further confirmation for the presence of abnormal growth in the colon using colonoscopy.

Author Contribution Statement

Conceptualization, GFH, RMS and MRAH; Data curation, YZN, SK, MIM, CHH, CKL, PR, II and HKC; Formal analysis, MAMS, YZN, HKC; Methodology, MAMS, GFH, RMS, II, HKC and MRAH; Supervision, GFH, RMS and MRAH; Writing – original draft, MAMS, YZN and HKC; Writing review & editing, MAMS, HKC and MRAH. All authors read and approved the final manuscript.

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Ethical approval

The ethics approval was granted by the Medical Research Ethics Committee (MREC) of the Ministry of Health Malaysia (NMRR-17-1830-36344).

Data Availability

The data are not publicly available due to privacy and ethical restrictions. The data presented in this study may be available conditionally from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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