RESEARCH ARTICLE

Editorial Process: Submission:06/01/2024 Acceptance:10/12/2024

Diagnostic Accuracy of Multitarget Stool DNA Test for Colorectal Cancer Screening and Detecting in Thailand

Varut Lohsiriwat, Aitsariya Mongkhonsupphawan, Pornraksa Ovartchaiyapong*

Abstract

Background: A multitarget stool DNA test offers high sensitivity and specificity for screening and detecting colorectal cancer (CRC) in Western populations. However, its accuracy in Asian people is not well known. This study aimed to examine the diagnostic performance of multitarget stool DNA tests in Thailand. **Methods:** A prospective cross-sectional study was conducted from January 2023 to November 2023 at a tertiary university hospital in Bangkok. The study included both asymptomatic and symptomatic patients who underwent stool DNA testing followed by colonoscopy. The multitarget stool DNA test targeted methylation statuses of SDC2, ADHFE1, and PPP2R5C genes. Sensitivity, specificity, and other diagnostic parameters were analyzed. **Results:** A total of 274 patients (mean age 62.1 years, 60.6% female) were enrolled. CRC was diagnosed in 17.2% of participants and 6.2% had advanced adenomas. The multitarget stool DNA test demonstrated a sensitivity of 91.5% (95% CI: 79.6-97.6) and specificity of 90.3% (95% CI: 85.7-93.8) for CRC detection. Its sensitivity for detecting CRC did not differ between right-sided lesions (92.3%) and left-sided lesions (91.2%) (P=0.901). The sensitivity for detecting CRC lesions size less than 2 cm was significantly lower than for larger lesions (25% vs 91.7%, p<0.001). Notably, the test's sensitivity and specificity for advanced colorectal neoplasms/cancer were 75.0% (95% CI: 62.6-85.0) and 91.9% (95% CI: 87.4-95.2), respectively. **Conclusions:** Multitarget stool DNA testing is highly sensitive and specific for CRC detection in Thai individuals. This testing could represent as a viable non-invasive alternative to colonoscopy especially in settings where colonoscopy is less accessible or less accepted by patients.

Keywords: Colorectal cancer- multitarget stool DNA test- screening- accuracy- Thailand

Asian Pac J Cancer Prev, **25 (10)**, 3661-3665

Introduction

Colorectal cancer (CRC) is a significant healthcare challenge and ranks as the third most prevalent cancer worldwide [1]. Compelling evidence supports the effectiveness of screening in reducing both the incidence and mortality of CRC [2]. While colonoscopy stands as one of the current standards for screening, its adoption is hindered by high costs, substantial workload and low patient compliance—particularly in resource-limited countries [3-5]. Fecal occult blood testing, as a non-invasive alternative, presents certain disadvantages, particularly its low sensitivity for detecting advanced adenomas (as low as 7%) and moderate sensitivity for detecting CRC (50% - 81%) [6, 7]. Additionally, there is a high false-positive rate attributable to non-human heme found in food and blood from the upper gastrointestinal tract in guaiac-based fecal occult blood testing [7].

Developed in recent years, the multitarget stool DNA test offers another non-invasive alternative for CRC detection. This test screens for the presence of DNA alterations released from tumor cells into the stool. Data primarily originating from the United States demonstrated a high sensitivity (93%) in detecting CRC and a 47% sensitivity in detecting advanced adenomas [8]. However, the number and specific target genes employed in the stool test may contribute to variations in test accuracy. Furthermore, the tumorigenesis of colorectal cancer may vary among racial and ethnic groups [9, 10]. To the best of our knowledge, no study has investigated the application of multitarget stool DNA for screening and detecting CRC in the population of Southeast Asia. The objective of this study is, therefore, to examine the diagnostic performance of multitarget stool DNA in Thailand.

Materials and Methods

Study design

In this prospective cross-sectional study, we studied the diagnostic performance of the multitarget stool DNA (COLOTECTTM, BGI Genomics, Shenzhen) for CRC and advanced adenoma detection using colonoscopy as the

Colorectal Surgery Unit, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. For Correspondence: quantu_p@hotmail.com

reference standard. Abnormal colonoscopic findings were confirmed by histopathology. Advanced adenoma was defined as an adenoma size ≥1cm or histology showing high-grade dysplasia or villous adenoma. Right-sided colon lesions were classified if located proximal to the splenic flexure. Left-sided colons lesion were located at or distal to splenic flexure up to 15 cm from the anal verge. Lesions below 15 cm from the anal verge was defined as rectal lesion. Notably, the multitarget stool DNA test in this study detects the methylation status of targeted genes, namely Syndecan-2 (SDC2), Alcohol dehydrogenase iron containing 1 (ADHFE1) and Protein phosphatase 2 regulatory B, γ(PPP2R5C). The test results were determined as positive if one or more genes were detected [11].

Setting and participants

All participants were enrolled from January 2023 to November 2023 at the Faculty of Medicine Siriraj Hospital, Bangkok, Thailand. Eligible subjects included both asymptomatic and symptomatic patients who underwent high-quality colonoscopy and were willing to collect stool sample for multitarget stool DNA testing.

Stool testing

Stool samples were obtained before mechanical bowel preparation. Neither diet nor medication restrictions were required. Approximately 2 g of stool was conserved in a preservative buffer and shipped to the laboratory within 7 days. All colonoscopies were performed by board-certified endoscopists who were blinded to the stool test results.

Sample size calculation and statistical analyses

Based on previous studies, the estimated sensitivity of the multitarget stool DNA test to detect colorectal cancer or high-grade dysplasia was 84% [8, 12]. The desired precision is 5%. The level of acceptable type I error was 5%. The calculated sample size was at least 207 participants [13].

Patients' characteristics were summarized as mean with standard deviation for continuous variables and as a number with a percentage for categorical variables. Sensitivity and specificity were estimated with corresponding 95% confidence intervals (95% CI) calculated using the exact binomial test.

Results

The results of the study were reported according to the STARD checklist [14]. The enrollment and outcomes were summarized in Figure 1. A total of 274 patients were analyzed with a mean age of 62.1 ± 10.0 years. Some 166 patients (60.6%) were female and 48 (17.5%) were symptomatic. Of the 48 symptomatic patients, lower gastrointestinal bleeding was the most common chief complaint (n=17, 35.4%) followed by bowel habit change (n=12, 25.0%) and anemia (n=6, 12.5%).

In this study, 47 patients (17.2%) were diagnosed with invasive cancer and 17 patients (6.2%) had advanced adenoma. Multitarget stool DNA test was positive in 65 participants (23.7%). The test results and colonoscopic findings are summarized in Table 1.

Multitarget stool DNA was positive in 43 of 47 patients with colorectal cancer – resulting in the test sensitivity and specificity of 91.5% (95% CI: 79.6 - 97.6) and 90.3% (95% CI: 85.7 - 93.8), respectively for colorectal cancer detection. For the 17.2% prevalence of observed colorectal cancer in this study, the positive predictive value, negative predictive value, accuracy and likelihood ratio are summarized in Table 2.

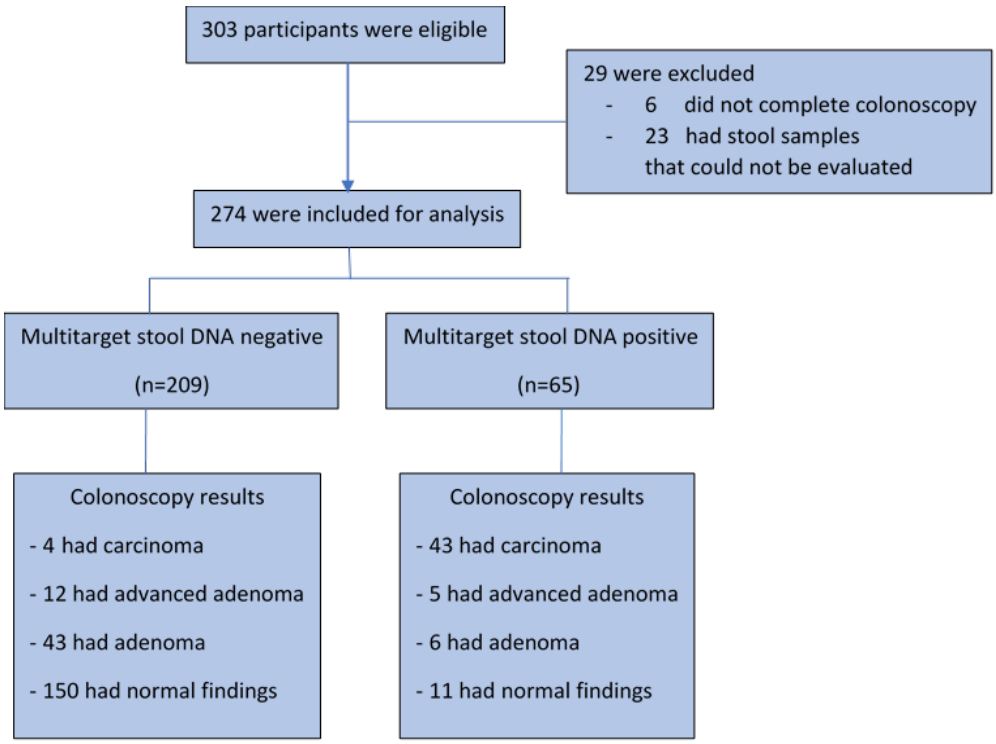

Figure 1. Enrollment and Outcomes

Table 1. Multitarget Stool DNA Test Results and Colonoscopic Findings

	Total Number	Carcinoma	Advanced adenoma*	Adenoma	Normal findings
Negative	209	4	12	43	150
Positive	65	43	5	6	11
ADHFE1 + PPP2R5C + SDC2	25	23	2	-	-
ADHFE1 + PPP2R5C	-	-	-	-	-
ADHFE1 + SDC2	3	3	-	-	-
PPP2R5C + SDC2	3	3	-	-	-
ADHFE1	8	2	2	1	3
PPP2R5C	4	2	-	-	2
SDC	22	10	1	5	6

Abbreviations: ADHFE1, Alcohol dehydrogenase iron containing 1; SDC2, Syndecan-2; PPP2R5C, Protein phosphatase 2 regulatory B, γ; *, Advanced adenoma was defined as an adenoma size ≥1cm or histology showing high-grade dysplasia or villous adenoma.

When combining patients with colorectal cancer or those with advanced adenoma together as individuals with advanced or invasive neoplasms, multitarget stool DNA identified 48 of 64 patients with these lesions. Accordingly, the test sensitivity and specificity were 75.0% (95% CI: 62.6 - 85.0) and 91.9% (95% CI: 87.4 - 95.2), respectively for advanced colorectal neoplasm/cancer. For the prevalence of observed advanced adenoma or invasive cancer of 23.4%, the positive predictive value, negative predictive value, accuracy and likelihood ratio are summarized in Table 2.

The sensitivity of the test for detecting colorectal cancer did not differ between right-sided colon (92.3%) and left-sided colon (91.2%) (P=0.901). Its sensitivity for detecting advanced adenoma was also comparable between right-sided lesions (70.6%) and left-sided lesions (76.6%) (P=0.624). The test had a 25% sensitivity to detect lesions less than 2 cm, but for lesions size of at least 2 cm the sensitivity of the test was high (91.7%) (P<0.001).

Discussion

This study demonstrated that multitarget stool DNA testing in Thai patients exhibited high sensitivity (91.5%), high specificity (90.3%) and high accuracy (90.5%) for detecting colorectal cancer. Additionally, the test had 29.4% sensitivity for detecting advanced adenoma with a high specificity (91.9%) and high accuracy (87.2%). Our results were comparable to those of multitarget stool DNA reports from the US [8, 12] – with a report of 92.3% and 42.4% sensitivity for detecting colorectal cancer and advanced adenoma, respectively.

The high sensitivity of test for CRC detection in our university-hospital-based study and multicenter US studies could be explained by the fact that multitarget stool DNA tests were used to cover common markers for CRC tumorigenesis such as NDRG4, KRAS and SDC2 [15]. Despite potential variations in tumorigenesis among racial and ethnic groups [9, 10], a stool DNA test with multiple gene targets remains effective and reliable for detecting invasive cancer. On the other hand, in a pooled sensitivity analysis of single-gene stool DNA test, the single-gene study had only 62.7% sensitivity for detecting CRC [16].

The ability of the multitarget stool DNA test to detect advanced precancerous lesions is not as good as that to detect CRC. This may be attributed to the lower shedding of neoplastic colonocytes in adenoma or a difference in tumorigenesis during the initial phase of premalignant transformation (adenomatous change), early malignancy and invasive cancer [17]. Currently, there are some combined DNA stool assays with fecal immunochemical test or fecal occult blood test aiming to improve their sensitivity to detect advanced adenoma or CRC [18]. Also, next-generation multitarget stool DNA test including high mutation frequencies such as TP53, APC and KRAS genes have recently become available to increase test sensitivity for screening and diagnosing CRC as well as monitoring CRC recurrence and predicting

Table 2. Multitarget Stool DNA Test Characteristic

Diagnostic parameters	Cancer Value (%) [95% CI]	Cancer or advanced adenoma+ Value (%) [95% CI]	Advanced adenoma Value (%) [95% CI]
Sensitivity	91.5 [79.6-97.6]	75.0 [62.6-85.0]	29.4 [10.3-56.0]
Specificity	90.3 [85.7-93.8]	91.9 [87.4-95.2]	91.9 [87.4-95.2]
Positive Likelihood Ratio	9.4 [6.3-14.2]	9.3 [5.8-14.9]	3.6 [1.5-8.6]
Negative Likelihood Ratio	0.1 [0.0-0.2]	0.3 [0.2-0.4]	0.8 [0.6-1.1]
Disease prevalence*	17.2 [12.9-22.2]	23.4 [18.5-28.8]	7.5 [4.4-11.7]
Positive Predictive Value*	66.2 [56.6-74.6]	73.9 [63.7-82.0]	22.7 [11.0-41.2]
Negative Predictive Value *	98.1 [95.3-99.2]	92.3 [88.7-94.9]	94.2 [92.2-95.6]
Accuracy*	90.5 [86.4-93.7]	88.0 [83.5-91.6]	87.2 [82.2-91.3]

*, These values are dependent on disease prevalence; +, Participants who had cancer or advanced adenoma (adenoma size ≥1cm or histology showing high-grade dysplasia or villous adenoma).

cancer prognosis [19]. With a next-generation stool DNA test, a recent multicenter study in US showed a 93.9% sensitivity for CRC and a 43.4% sensitivity for advanced precancerous lesions [20]. Some previous studies compared the performance of multitarget stool DNA tests to that of fecal immunochemical test (FIT) [8, 12, 20]. They reported that multitarget stool DNA had higher sensitivity in detecting invasive cancer or advanced adenoma but comparable or slightly lower specificity. These findings confirm that multitarget stool DNA is a viable option for stool-based CRC screening.

The size of the lesions could be another determining factor for predicting the accuracy of stool DNA test. Our study revealed only 25% sensitivity for detecting lesions less than 2 cm – whereas 91.7% sensitivity was seen in lesions of at least 2 cm. These findings were consistent with other reports which demonstrated an increase in sensitivity as colonic lesions grow larger [12]. Thus, our findings again highlight the limitation of stool DNA tests in detecting small lesions. Partly due to this limitation, many societies recommend retesting every 1-3 years [21-23]. Meanwhile, the optimal interval of stool DNA testing requires further studies.

The sensitivity to detect proximal and distal colonic lesions was comparable in our study (92.3% vs 91.2%, respectively). These findings were somehow different from those reported from a large multicenter study from US – in which a lower sensitivity was reported for detecting proximal precancerous lesions (33.2%) but it remained high (approximately 90%) for invasive proximal lesions [8]. The discrepancy between the two studies may be attributed to the inclusion of symptomatic patients in our series whereas the other study did not. As a result, there was a larger number of invasive cancers compared to advanced precancerous lesions in the proximal colon in our series.

Not only is high sensitivity tremendously important for cancer screening tests, but good specificity is also crucial. In our study, the test demonstrated high specificity, with rates of 89.9% for detecting invasive cancer and 91.9% for detecting advanced adenoma. However, there were a few participants with false positive results, which may have occurred due to several factors. Firstly, non-advanced adenomas may have caused the positive test results and were subsequently included in the negative arm based on colonoscopic and histologic findings. Additionally, other gastrointestinal cancers besides colorectal cancer may shed neoplastic cells into the stool. Unfortunately, there are no specific recommendations for further investigation in such situations [24]. Another possible cause was missed lesions - although in our view it is unlikely as the colonoscopy was performed by highly experienced endoscopists working in a World Gastroenterology Organization (WGO)-accredited endoscopic training center.

Our study had some limitations despite our relatively modest sample size. First, we did not exclusively focus on the ability of multitarget stool DNA test for CRC screening as found in several published studies. In fact, we intentionally included both symptomatic patients and average-risk individuals to enhance the relevance of the study in daily practice. Second, data on the pathologic staging of those diagnosed with CRC were not available at this moment because some patients underwent surgery in other hospitals or remained on the waiting list for operation or, even, deny undergoing surgery.

In conclusion, this study in the Thai population demonstrated that the multitarget stool DNA test is an effective option for screening colorectal cancer, with very high sensitivity (91.3%) and specificity (89.9%) for detecting invasive lesions, and optimal sensitivity (74.6%) for detecting advanced precancerous lesions. It could serve as a viable alternative to the FIT or invasive colonoscopy for CRC detection.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

Funding Statement

The present study was funded by the Faculty of Medicine, Siriraj Hospital

Ethical Declaration

The study protocol was reviewed and approved by the Siriraj Institutional Review Board. Certificate of Approval number is Si 340/2023.

Availability of Data

Data will be available upon reasonable request.

Conflict of Interest

All authors declare that they have no conflict of interest.

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