RESEARCH COMMUNICATION

Screening for Colorectal Neoplasias with Fecal Occult Blood Tests: False-positive Impact of Non-Dietary Restriction

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Abstract

Objective: Screening for colorectal cancer using guaiac-based fecal occult blood tests (gFOBT) is well established in Western populations, but is hampered by poor patient compliance due to the imposed dietary restrictions. Fecal immunochemical tests (FIT) do not require dietary restriction, but are more expensive than gFOBT and therefore restrict its use in developing countries in Asia. However, Asian diets being low in meat content may not require diet restriction for gFOBT to achieve equivalent results. The objective of this study was to evaluate and compare the validity and suitability of gFOBT and FIT or a combination of the two in screening for colorectal neoplasias without prior dietary restriction in an Asian population. Methods: Patients referred to the Endoscopic Unit for colonoscopy were recruited for the study. Stool samples were collected prior to bowel preparation, and tested for occult blood with both gFOBT and FIT. Dietary restriction was not imposed. To assess the validity of either tests or in combination to detect a neoplasm or cancer in the colon, their false positive rates, their sensitivity (true positive rate) and the specificity (true negative rate) were analyzed and compared. Results: One hundred and three patients were analysed. The sensitivity for picking up any neoplasia was 53% for FIT, 40% for gFOBT and 23.3% for the combination. The sensitivities for picking up only carcinoma were 77.8% , 66.7% and 55.5%, respectively. The specificity for excluding any neoplasia was 91.7% for FIT, 74% for gFOBT and 94.5% for a combination, whereas for excluding only carcinomas they were 84%, 73.4% and 93.6%. Of the 69 with normal colonoscopic findings, FOBT was positive in 4.3%, 23.2 %and 2.9% for FIT, gFOBT, or combination of tests respectively. Conclusion: FIT is the recommended method if we are to dispense with dietary restriction in our patients because of its relatively low-false positivity and better sensitivity and specificity rates.

Keywords: Colorectal cancer - fecal occult blood test - screening - diet restriction

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Introduction

There is a reducing trend in the incidence of colorectal cancer (CRC) in the West (Greenlee et al., 2001) but in Malaysia CRC still forms 13.2% of all cancers and is the commonest cancer in males. In 2006, 2866 cases were registered nationally (Kong et al., 2010; Ministry of Health Malaysia, 2006) and there may be a rising trend. The prognosis of the disease is good if the disease is diagnosed early (Ciccolallo et al., 2005) but unfortunately, less than 40% of CRC are diagnosed at a local stage; the rest are either regionally advanced or at the metastatic stage at the time of diagnosis (Greenlee et al., 2001; Kong, et al., 2010). Although the overall five year survival rate is 60% for all stages, data from the West shows that deaths from colon cancer are decreasing at the rate of 1.9% per year (Landis et al., 1998). There is circumstantial evidence that this decrease may be due to an active screening program.

In a large case control trial, a biennial screening program was shown to reduce the mortality from CRC (Kronborg et al. , 1996). In addition, Hardcastle et al., 1989 showed that 56% of cancers in the screened group were at the early stage as opposed to only 10.6% in the control group. Furthermore, to depend on early presentation of symptoms as a diagnostic strategy is of no benefit because 85% of symptomatic patients would have established disease already at the time of presentation (Silverman et al., 1988).

There are two categories of screening tests for colorectal cancer. Structural examinations e.g. colonoscopy, are better at detecting pre-malignant conditions, and offer the opportunity for prevention of CRC. Notwithstanding their reliability, they are not practicable in a developing country, being expensive, manpower dependent, and has some degree of morbidity. Fecal tests are relatively cheap and more practical for population screening. The best studied tests rely on detection of fecal occult blood (FOBT). Of these, there are two main methods available, guaiac tests (gFOBT) and the newer fecal immunochemical tests (FIT).

Of these, gFOBT is the most common test in use and the only CRC screening test for which there is evidence

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of efficacy from prospective, randomized controlled trials (Hardcastle, 1996; Kronborg , 1996; Mandel, 1993). The gFOBT is based on the principle that it detects any pseudoperoxidase activity in the feces. Heme either as intact hemoglobin or free heme has pseudoperoxidase activity. Therefore, in the presence of heme and a developer (hydrogen peroxide) guaiac acid is oxidized, producing a blue color. The detection of this blue color indicates a positive gFOBT. But because heme is present in red meat and peroxidase activity is present in some fresh fruits and vegetables, dietary restriction is recommended for three days prior to testing in order to avoid false positives.

FIT on the other hand, uses monoclonal and polyclonal antibodies to detect the intact globin protein portion of human hemoglobin. The labeled antibody attaches to the antigens of any human globin present in the stool resulting in a positive test. Globin does not survive passage through the upper gastrointestinal tract. Therefore, FIT detecting globin is specific for occult bleeding from the large bowel. Furthermore, because FIT does not react with non-human globin, dietary restriction is not necessary when screening with these tests. However, the test is more expensive. An evaluation of its use by (Levi, et al., 2007) revealed a sensitivity of 94.1% and specificity of 87.5%. respectively for cancer and 67% and 91.4% respectively, for any clinically significant neoplasia.

The Malaysian diet is unique in that the proportion of meat is much less than that of Western diets. Furthermore, dietary restriction, in our experience, is difficult to enforce in our patients. Clearly there are problems with either tests. While gFOBT is well-tested, cheaper and more appropriate for Malaysia as a screening test because of the large numbers involved, its need for dietary requirements is a problem. FIT on the other hand, does not require dietary restriction, is more specific but it is costly. Furthermore, there have not been any good randomized controlled trials assessing its validity.

The purpose of the present study was to compare and evaluate the validity and suitability of these two screening tests in our population in whom dietary restriction was dispensed with.

Materials and Methods

UMMC Ethics Committee approval (Ethics committee IRB reference number : 482.3) was obtained prior to the study commencement, and each patient provided written informed consent. Patients referred to the Endoscopic Unit for colonoscopy and from July 2007 to June 2008 were recruited for the study. The indications for the colonoscopy are summarized in Table 1. Patients with active haematochezia or frequent haematochezia were excluded. Patients were not required to undertake any dietary restriction or to refrain from any medication. Stool samples were obtained when the patient was first seen in clinic. The stool samples were immediately tested for occult blood with both the gFOBT (Three Field Hemdetect, Dipromed, Germany) and FIT (OC-Light, Eiken, Japan) .

Fecal Occult Blood Tests

For gFOBT, the stool samples were applied to the 3 field test card. The test cards were not rehydrated. Two drops of developer (hydrogen peroxide) were applied to each of the 3 test slide window on the test card and one single drop on the control field. A test was considered positive if any blue color appeared in the test slide window within 20 to 120 seconds after the addition of developer. For FIT, each test kit comes with a plastic applicator attached to the lid of the kit bottle, test solution containing anti-human hemoglobin antibody and a test strip. Stool samples were transferred to the applicator and dipped into the test solution, the bottle secured and then shaken. The lower tip of the chromatography test strip is then dipped into the resulting suspension. If human hemoglobin is present, it binds with the anti-human hemoglobin and the resulting complex migrates chromatographically along the test strip to the test line, which appears as the second blue line. Approximately 90 seconds is required for the test to develop. The test is considered positive if blue lines appeared at the lower and the upper centers of the strip. The appearance of single blue line (control line) at the upper end of the strip is considered a negative result. Absence in both lower and upper centre of the strip or only at the lower centre of the strip is considered an equivocal result. For the purpose of study, only positive results were considered.

Colonoscopy

After standard bowel preparation, all patients underwent colonoscopy with visualization of the entire colon including the caecum. Colonoscopy was performed within 8 weeks from the date of stool sample collection. Patients with incomplete examination or poor bowel preparation were excluded from analysis. The colonoscopies were performed by an endoscopist who was blinded to the FOBT tests. Any obvious or suspected lesions were biopsied and sent for histology. For the purposes of the analysis, either a carcinoma or an adenoma was considered to be a neoplastic lesion. Patients with diverticula or hyperplastic polyps were classified as normal.

Analysis

To assess the validity of either tests or in combination to detect a cancer or neoplasm in the colon, their overall `pick-up rate', their false positive rates, their false negative rates and their sensitivity (true positive rate) and the specificity (true negative rate)were analyzed and compared.

Results

One hundred and thirty-four patients were enrolled in this study and of these, 103 patients were eligible for analysis. The others were excluded because they did not fulfill one or more of the inclusion criteria. The demographic characteristics of the patients are summarized in Table 2. Most of the patients were in the 6th decade, with males and Chinese forming the majority.

Table 1. Indications for Colonoscopy

Indication	N (%)
Altered bowel habit	40 (38.8)
Abdominal pain	9 (8.7)
Abdominal mass	2(1.9)
Post colonic surgery surveillance	15 (14.6)
Polyp follow-up surveillance	18 (17.5)
Screening	7 (6.8)
Anemia	4 (3.9)
Family History	4 (3.9)
Others	4 (3.9)

Table 2. Demographic Characteristics of Patients

Characteristic	N (%)
Age in years (mean ± SD)	61.2 ± 10.6
Male	57 (55.3)
Female	46 (44.7)
Malay	14 (13.6)
Chinese	58 (56.3)
Indian	29 (28.2)
Others	2 (1.9)

Table 3. Positivity rate of Fecal Occult Blood Test by Colonoscopic Findings

		Fecal occult blood test		
Finding	N (%)	FIT(%)	gFOB(%)	FIT+gFOBT(%)
Normal	69 (67.0)	4.3	23.2	2.9
Adenoma	21 (20.4)	42.9	28.6	9.5
Carcinoma	9 (8.7)	77.8	66.7	55.6
Colitis	3 (2.9)	66.7	66.7	33.3
Ulcer	1 (1)	100	100	100

Normal includes patients with diverticulosis or haemorrhoids; FIT, fecal immunochemical test; gFOBT, guaiac FOBT

Table 4. Sensitivity and Specificity of Fecal Occult Blood Tests (FOBT) with (95% Confidence Intervals)

	Fecal Occult Blood Test		
	FIT	gFOBT	FIT+ gFOBT
Sensitivity (%)			
Carcinoma	77.8 (40.2 – 96.1)	66.7 (30.9 -90.9)	55.5 (22.6 – 84.6)
All neoplasias*	53.3 (34.6 - 71.2)	40 (23.2 - 59.2)	23.3 (10.6 - 42.7)
Specificity (%)			
Carcinoma	84 (74.0 - 90.0)	73.4 (63.1 - 81.7)	93.6 (86.1 - 97.4)
All neoplasias	91.7 (82.3 - 96.6)	74 (62.2 - 83.2)	94. 5 (85.8 - 98.2)

*Adenomas and carcinomas; FIT, fecal immunochemical FOBT; gFOBT, guaiac FOBT

Overall 'pick-up' or positivity rate of occult blood

The results are summarized in Table 3. Of interest, was the positivity rate in those with normal colonoscopic findings i.e. the false positivity rate. There was a significant difference in the positivity rate between the three modes of analysis in the 69 patients with normal colonoscopic findings; the gFOBT detecting occult blood in nearly a quarter of these patients (Table 3). FIT by itself or a combination of the two revealed less occult blood in this group of normal findings, the combination of the two tests doing best.

Furthermore, the FIT by itself appeared to detect occult blood in patients with neoplasms more often than gFOBT or even a combination of the two (Table 3). The patients with colitis or ulcers were too few to make meaningful conclusions.

Overall, the ability to pick up either neoplasms or carcinoma correctly i.e. the sensitivity, was best with FIT (Table 4). However their 95% confidence intervals were relatively wide. A combination of both the FIT and gFOBT did not increase the sensitivity. Furthermore, between the two tests, the ability to exclude either a neoplasm or a carcinoma correctly i.e. the specificity was best with FIT. A combination of both the test provided marginal improvement in the specificity (Table 4).

Discussion

We set out to compare the validity of FIT, gFOBT or a combination of the two for detecting neoplasms of the colon and in patients in whom dietary restrictions were dispensed with. Further, we decided to explore the option of non-dietary restriction in our study because we felt that it may improve participation. This has been borne out in a Caucasian population; when dietary restriction was removed (Cole et al., 2003) the participation rate improved by 28%.

FIT fared the best, giving the best sensitivity and specificity. Furthermore, its positivity rate was least in patients with normal colonoscopic findings - a reflection of its low false positivity in this group of patients. On the other hand, gFOBT tended to pick more `occult blood' in patients with normal colonoscopic findings, indicating a relatively high false positivity in patients without dietary restrictions. The proposed theory that an Asian diet being low in ` red-meat' content would make gFOBT as good as FIT is therefore not supported. The most appropriate test would be FIT especially in our set of patients in whom dietary restriction is difficult to enforce because it had relatively, the best sensitivity and specificity. Furthermore, the additional direct cost of FIT compared to gFOBT would be counter-balanced by the reduced need for unnecessary follow-on colonoscopies (thereby reducing overall cost) in those patients who would have had false-positive gFOBT results

We also explored the possibility that a combination of both tests would be more valid as opposed to either of the tests. A combination produced better specificity, but a decrease in sensitivity. A higher specificity for the combination are consistent with other studies (Allison, et al., 1996; Greenberg, et al., 2000) in whom diet restriction was enforced albeit they had a higher specificities; around 96% for either neoplasms or cancer. The most plausible explanation for the high specificity was that the FIT counteracted the specificity of the gFOBT while maintaining its high specificity.

Most of the other studies on the validity of FOBT have been confined to Western population. To the best of our knowledge this is the first of its kind to be done in a

multi-ethnic Asian population such as seen in Malaysia. Furthermore, its newness lies in attempting to assess these tests in patients in whom dietary control was dispensed with, which realistically, mimics the likely scenario in population screening with poor patient compliance to dietary restrictions.

Generally, our results appear to be confirmatory of other studies comparing the two methods albeit, they had no dietary restriction. Allison et al. 1996 in a large population based survey involving more than 8000 patients showed that tests for occult blood, irrespective of the kind of tests, show better specificity rather than sensitivity. Notwithstanding, FIT tended to fare better, displaying sensitivity of 68% and specificity of 97%, compared to 37% sensitivity and 86% specificity for gFOBT. A combination of the two tests showed only a marginal increase in sensitivity or specificity. The low sensitivity of FIT has been borne out in other large surveys. In a screening involving more than 21,000 patients (Morikawa et al., 2005) a sensitivity of only 27.1% for detecting advanced neoplasia and 65.5 % for invasive cancers was reported. In another study where the two tests were compared for patients undergoing colonoscopy for gastrointestinal bleeding, Gopalswamy et al., 1994 showed that FIT had better sensitivity to gFOBT but less specificity.

The US and European experience have indicated that the prognosis of colorectal cancer is generally good if the disease is detected early (Gatta et al. 2003; Ciccolallo et al., 2005). This underscores the importance of screening, provided that it does indeed detect early cancers. There is evidence that it does so. In a large prospectively controlled trial of responders and non-responders and involving more than 107,349 patients without symptoms of colorectal cancer, Hardcastle et al. 1989 showed that of the 618 cancers in the screened group 52% were at Stage 1while in the control group, of the 123 cancers detected, 10.6% were at Stage 1. The authors concluded that colon cancers detected by screening were at a less advanced stage but it was` too early to show any effect of screening on mortality from colorectal cancer'. However, a more recent report (Espey et al., 2007) suggests that screening for colorectal cancer had some effect on reducing mortality.

The limitations of our study are recognized. The sensitivity of a single gFOBT can vary with the method of collection. A thick stool smear, off-white discoloration of the smeared paper, drying out of specimen, and exposure to a high ambient temperature can each result in a false-negative test. In our study the gFOBT was not rehydrated and only a single stool sample was used for testing. This may explain the discrepancies with other worker's results. There has been scepticism about the use of a single finger analysis as opposed to a 6 sample home based FOBT. Collins et al. 2005 compared a one sample test with the recommended 6 sample test and found a sensitivity of 4.9% in the single sample and 23.9 % in the six sample tests. But the specificities were only marginally different i.e. 93.9 and 97% % respectively. Admittedly however, compliance for a 6 sample stool may be difficult to enforce. The colonoscopies in our patients were done within 8 weeks of the stool tests. This may be too long an interval but there were constraints of manpower and long waiting lists. Lastly, our results may not be generalizable to an asymptomatic population because our group of patients were those in whom an endoscopy was indicated i.e. they were symptomatic. Notwithstanding, the main thrust of our study was to compare the two tests.

In concluding, gFOBT was associated with an increased false positivity rate in patients who did not undergo dietary restriction, despite having a diet with low red meat content. FIT was unaffected by this non-restriction. For the present, therefore, FIT is recommended for population screening in Malaysia as uptake is likely to be better and overall costs lower. More research is needed and in a general population setting to determine the most cost-effective strategy for CRC screening.

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