Short Communication

Transferrin Dipstick as a Potential Novel Test for Colon Cancer Screening: A Comparative Study With Immuno Fecal Occult Blood Test

Jian-qiu Sheng,[1] Shi-rong Li,[1] Zi-tao Wu,[1] Chang-hong Xia,[1] Xia Wu,[1] Jigui Chen,[2] and Jianyu Rao[3]

[1]Department of Gastroenterology, Beijing Military General Hospital, Nanmenchang 5#, Dongcheng District, Beijing, People's Republic of China; [2]The Eight Hospital, Wu Han City, Hubei Province, People's Republic of China; and [3]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, and Department of Epidemiology, School of Public Health, University of California, Los Angeles, California

Abstract

Recent proteomic studies identified Transferrin (Tf) as a potential biomarker for cancer. We examined the efficacy of the newly developed Tf dipstick for detecting colorectal cancer and premalignant lesions, and compared that to Immuno Fecal Occult Blood test (IFOBT). Fecal samples from 110 patients including 40 colorectal cancer, 36 premalignant subjects (including 16 with high-risk adenomas and 20 with ulcerative colitis), and 34 low-risk subjects were collected before colonoscopic examination. Compared with IFOBT, Tf had a significantly higher positive rate in patients with colorectal cancer and premalignant lesions (76% for Tf versus 61% for IFOBT, respectively; $\chi^2 = 4.38$; $P < 0.05$). The difference of positivity was mainly observed in patients with premalignant lesions (72% for Tf versus 44% for IFOBT; $\chi^2 = 5.71$; $P < 0.05$), whereas the positive rates in cancer group and in low-risk group were similar (both $P > 0.05$). Combining Tf with IFOBT together (either/or) had 90% positive rate in cancer patients, 78% in premalignant patients, and 29% in low-risk subjects. The overall accuracy of IFOBT and Tf tests for detecting colorectal cancer and premalignant lesion was 69.0% and 76.4%, respectively. Tf dipstick test seems to be a highly sensitive test for detecting not only cancer, but also premalignant lesions, and provides an additional tool for colorectal cancer screening.(Cancer Epidemiol Biomarkers Prev 2009;18(8):2182–5)

Introduction

In the United States, colorectal cancer is the third most common cancer in this country in both males and females, with ~50,000 new cases diagnosed and over 50,000 deaths in 2008 alone (1). Although screening and detection of early stage cancer reduce the mortality of colon cancer, the overall screening rate in this country is <50% in adults over ages 50 years (recommended screening population; ref. 2). Fecal occult blood tests, either the traditional chemical based (Guaic test) or more recent antibody based (Immunofecal Occult Blood Test, IFOBT), are quite effective screening tool for colon cancer (3, 4). However, even with repeat test of multiple samples, significant number (about 15-30%) of colon cancers, especially the early stage cancers and premalignant adenomas (up to 50%), may be missed using these methods (5). Therefore, new tests are needed to increase the sensitivity of the screening.

In this study, we did a preliminary examination of the novel Transferrin (Tf) dipstick test for colon cancer screening. Tf is a type of β_1 globulin with a molecular weight of 77 KD, and the main function of the protein is to transport extracellular iron into cells through membrane receptor–mediated endocytosis (6). As in the case of hemoglobin, serum Tf may be leaked into gastrointestinal tract during the course of bleeding. However, compared with hemoglobin, Tf is more stable (7, 8). Therefore, it is potentially a more sensitive test than hemoglobin-based test. Although the functional role of overexpression of Tf receptor has been extensively studied in cancer (for review, see ref. 9), recently, a number of proteomic studies showed that increased Tf level in serum and body fluid in a number of cancer types, including colon cancer (10, 11). These findings provide the rationale for developing a simple dipstick test that may be used as a tool for cancer screening. This study was done to preliminary test the hypothesis.

Materials and Methods

Materials. Both IFOBT and Tf test kits were kindly provided by WHPM, Inc., El Monte, CA.

Patient Population. A total of 110 patients were recruited in a continuous fashion in Endoscopic Center of Beijing Military General Hospital from January 2008 to April 2008. The inclusion criteria were as follows: age over 20 y, male or female, and with Chinese citizenship. Patients with age <20 y and non-Chinese were excluded.

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Requests for reprints: Jian-qiu Sheng, Department of Gastroenterology, Beijing Military General Hospital, Nanmenchang 5#, Dongcheng District, Beijing, China, 100700. E-mail: Jianqiu@263.net or Jianyu Rao, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, and Department of Epidemiology, School of Public Health, University of California, Los Angeles, CA. Phone: 310-794-1567; Fax: 1-310-825-7795. E-mail: jrao@mednet.ucla.edu

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Table 1. Patient demographics in colorectal cancer, high-risk, and low-risk subjects

	Colorectal cancer (40)	Premalignant (36)	Low risk (34)
Age			
Mean	63	60	61
Range	38-81	26-84	35-82
Sex			
Male	26	24	20
Female	14	12	14
Duke's Stage			
A	4		
B	17		
C	13		
D	6		
Grade			
Well	14		
Moderate	16		
Poor	10		

All subjects underwent standard colonoscopic examination. Among them, 40 subjects were found to have colorectal cancer (with histologic confirmation), 36 with premalignant lesions, including 16 subjects with high-risk adenomas (villous adenoma, adenoma with moderate-severe dysplasia, multiple adenoma or adenoma of ≥1 cm), and 20 subjects with ulcerative colitis, and 34 with either small single adenoma (<0.5 cm, 8 cases) or no abnormalities (26 cases).

Fecal Sample Collection and IFOBT and Tf Analysis. One fecal sample was collected from each patient. Fecal samples were collected and processed according to the manufacture's instruction (WHPM, Inc.). All fecal samples were collected before endoscopic examination. The detail instruction of both Tf and IFOBT tests can be found in the manufacture's insert (WHPM, Inc.). Briefly, after applying the fecal sample on the strip, the result were interpreted within 5 min (the result was invalid after 5 min). A red bar in control area (C) only was considered as negative. A red bar in testing area (T) and control area (C), respectively, was considered as positive. The test was considered invalid if there was no red bar in control area (C). To prevent the prezone phenomenon, for all negative IFOBT samples, repeat tests were done after diluting the original fecal suspension according to the manufacture's instruction. Only repeat test negative samples were considered as true negative.

Statistical Analysis. The positive rate of IFOBT alone, Tf alone, and IFOBT combined with Tf (IFOBT + Tf) was calculated. χ^2 and McNemar's test were done to determine the significant level of difference. Statistical significance was established at a P value of <0.05.

Results

Table 1 presents the overall demographic information in the three groups of subjects examined. There was no significant difference in age and sex composition of subjects in colorectal cancer group, premalignant lesion group, and low-risk group examined.

Table 2 shows the positive rate of Tf alone, IFOBT alone, and combined Tf and IFOBT (either/or scenario) in fecal samples collected from the three groups of patients. The positive rate of IFOBT test alone was 75%, 44%, and 12%, respectively, in cancer, premalignant lesion, and low-risk patients. For Tf, the positive rates in cancer and premalignant lesions were similar (80% and 72%, respectively; $\chi^2 = 0.63$; $P > 0.05$), and in low-risk subjects, the positive rate was 24%. Compared with IFOBT, Tf had a significantly higher positive rate in patients with colorectal cancer and premalignant lesions (76% versus 61%, respectively; $\chi^2 = 4.38$; $P < 0.05$). The difference of positivity was mainly observed in patients with premalignant lesions (72% versus 44%, respectively; $\chi^2 = 5.71$; $P < 0.05$), whereas the positive rates in patients with cancer and in low risk group were similar (the χ^2 value is 0.29 and 1.62, respectively, both $P > 0.05$). Combining Tf and IFOBT together in either/or scenario further increased the positive rate in cancer group to 90%, premalignant lesion group to 78%, and the positive rate in low-risk subjects was 29%. The Overall accuracy of IFOBT and TF tests for detecting colorectal cancer and premalignant lesion was 69.0% versus 76.4%, respectively.

Table 3 presents the concordance of the two tests in three groups of patient samples. Again, no significant differences were seen in colorectal cancer and low-risk groups. However, in premalignant group, 12 of 36 samples (33.3%) were positive for Tf test but negative by IFOBT test. In contrast, only 2 of 36 samples (5.6%) were positive by IFOBT but negative by Tf test. The difference reached statistical significance ($P < 0.05$ by McNemar's test).

Discussion

Fecal occult blood test, especially IFOBT, is a well-accepted effective screening method for colorectal cancers (12-14). The main detection target in IFOBT is fecal hemoglobin. IFOBT has a reasonable sensitivity, especially

Table 2. Positive rate of Tf alone, IFOBT alone, and Tf + IFOBT in fecal samples from colorectal cancer patients, premalignant subjects, and low-risk subjects (n = 110)

Diseases	n	IFOBT		Tf		Tf + IFOBT	
		+	−	+	−	+	−
Colorectal cancer	40	30 (75%)	10 (25%)	32 (80%)*	8 (20%)	36 (90%)*	4 (10%)
Premalignant subjects†	36	16 (44%)	20 (56%)	26 (72%)*,‡	10 (28%)	28 (78%)*,‡	8 (22%)
Low risk population§	34	4 (12%)	30 (88%)	8 (24%)	26 (76%)	10 (29%)	24 (71%)

*$P < 0.01$ comparing to low-risk population by χ^2 test.
†Includes 16 subjects with high-risk adenomas (villous adenoma, adenoma with moderate-severe dysplasia, multiple adenoma or adenoma ≥1 cm) and 20 subjects with ulcerative colitis.
‡$P < 0.05$ comparing to IFOBT alone by χ^2 test.
§Includes 8 subjects with small single adenoma (<0.5 cm) and 26 healthy subjects.

Table 3. Concurdance of IFOBT and Tf tests in fecal samples from colorectal cancer patients, premalignant subjects, and low-risk subjects (n = 110)

	Tf (+)	Tf (−)	Total
A. Colorectal cancer (n = 40)			
IFOBT (+)	26 (65.0%)	4 (10.0%)	30
IFOBT (−)	6 (15.0%)	4 (10.0%)	10
Total	32	8	40
B. Premalignant lesions (n = 36)*			
IFOBT (+)	14 (38.9%)	2 (5.6%)	16
IFOBT (−)	12 (33.3%)	8 (22.2%)	20
Total	26	10	36
C. Low risk (n = 34)			
IFOBT (+)	2 (5.0%)	2 (5.0%)	4
IFOBT (−)	6 (15.0%)	24 (60.0%)	30
Total	8	26	40

*$P < 0.05$ by McNemar's test.

compared with chemical based assay (Guaiac test; ref. 14). However, the IFOBT test is far from perfect, and the false-negative rate range from 15% to 30%, which may vary by the number of fecal samples tested and from manufacture to manufacture (15).

With tremendous resources devoted to improve the screening of cancers, especially colon cancers, little progress has been made. There also has been specific effort to improve the FOBT test itself over the years with only limited success. For example, a "hydrated fecal occult blood test" was developed to improve the sensitivity of Guaiac test, but it was then eliminated due to its high false-positive rate (16). Other biomarkers, such as "fecal microalbumin (17)," "fecal decay-accelerating factor (18)," "fecal calprotectin (19)," and "fecal tumor associated antigen (20)" have also been studied but without significant improvement of detecting colon cancer. IFOBT certainly offers several advantages over conventional chemical-based assay, including higher sensitivity, not required to restrict certain food types that may cause false positiveness, etc. However, the inherent limitation of IFOBT is the fact that the test is designed to detect hemoglobin, which by itself may not be stable and not cancer specific.

Tf is a type of β1 globulin, with a molecular weight of 77 KD, accounts for about 0.3% to 0.5% of the total plasma protein. It is mainly synthesized in liver, and the main function of the protein is to transport extracellular iron into cells through membrane receptor-mediated endocytosis (6). Each Tf molecular can bind two iron atoms. Because many intracellular enzymes such as ribonucleotide reductase all require iron as an agon, Tf is extremely essential for cell growth and survival. Similar to hemoglobin, serum Tf may be also leaked into gastrointestinal tract and then discharges into the feces with gastrointestinal bleeding diseases. However, unlike hemoglobin, Tf is resistant to the effects of digestive enzymes and bacteria. Thus, Tf is more stable than hemoglobin in feces and can potentially be a more sensitive marker to detect gastrointestinal bleedings, including cancer. On the other front, proteomic studies consistently identified Tf as a marker with altered expression in a number of cancer types (10, 11). These findings prompted us to test the newly developed Tf dipstick in actual patient samples, using fecal sample for colon cancer detection as a starting point because the clear clinical need.

The results of this preliminary study show that Tf had a higher positive rate in cancer and premalignant high-risk lesions than that of IFOBT, and the difference seems to be mostly due to the higher positive rate in premalignant lesions in Tf over IFOBT. However, although not statistically significant, we found more "false" positive findings in low-risk patients (8 of 34 for Tf versus 4 of 34 for IFOBT). It should be noted that in our preliminary study, we also evaluated patients with upper gastrointesntinal bleeding, including cancers of esophageal and stomach, and found that Tf can detect in ~40% of upper gastrointestinal bleeding (data not shown). It is possible that some of the eight low-risk subjects with positive findings might have conditions associated with upper gastrointestinal bleeding that was undetected. In addition, in this study the Tf and IFOBT tests were done only once for each sample; therefore, the reproducibility of the tests cannot be determined. However, according to manufacture's unpublished report, the reproducibility of these tests reaches 95% on same sample setting.

Although the results of the study might be limited by relatively small sample size, this was the first study to show a marker identified by proteomic means that can be actually converted into a simple dipstick-based test and potentially used for screening of cancers. Similar approach may be used for other identified markers as well. Together, Tf dipstick may provide a simple alternative for cancer screening not only in colon, but other cancer types as well, and large-scale studies are warranted to further examine the efficacy of the test.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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