Clinico-pathological Characteristics of Colorectal Cancer with a False-negative Result on Immunochemical Occult Blood Screening

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Source of Financial Support

The present study was supported in part by Grants-in-Aid for Cancer Research (No.8-8) from The Ministry of Health and Welfare of Japan.

Abstract

Background/Aims: To clarify the clinico-pathological features of colorectal cancer showing negative results on an immunochemical fecal occult blood screening.

Methodology: The study subjects included 36 colorectal cancer cases diagnosed as false-negative (negative group) and 202 colorectal cancer cases diagnosed as true-positive (positive group) in cancer screening by the immunochemical fecal occult blood test. They were compared in terms of their individual factors, such as site, size, Dukes' classification and histological type of their cancer lesions.

Results: The prevalence of rectal cancers was higher in the negative group than in the positive group ($p<0.05$), but there were no differences between the two groups for any other factors.

Conclusion: These findings suggest that immunochemical fecal occult blood testing is inferior for the detection of rectal cancer.

Key words: Colorectal cancer, False-negative, Immunochemical fecal occult blood screening.

Introduction

Recently in Japan, several types of immunochemical fecal occult blood test kits for colorectal cancer screening, such as enzyme-linked immunosorbent assays, reversed passive hemagglutination, latex agglutination, and immunocoloric method have been developed (1-3). The author previously reported on high diagnostic accuracy of a immunochemical fecal occult blood test for colorectal cancer, compared with a conventional chemical test, Hemoccult II, based on the results in an actual clinical setting or through actual screening testing (4).

So far, however, low diagnostic accuracy of chemical fecal occult blood test for colorectal cancer has been reported (5). In the present study, in an attempt to clarify the features of colorectal cancer that give negative results on immunochemical fecal occult blood test, the author investigated their clinico-pathological characteristics by comparing them with those of colorectal cancer with positive results.

Patients and Methods

A comparative study was conducted between the false-negative and true-positive groups in the colorectal cancer screening. The patients who showed negative results on the immunochemical fecal occult blood test at the time of colorectal cancer screening, and were diagnosed as having colorectal cancer within one year after the screening, were defined as false-negative patients. In contrast, those who showed positive results on the immunochemical fecal occult blood test at the time of screening, and were diagnosed as having colorectal cancer by the subsequently performed

close examination, were defined as true-positive patients.

Based on the above definitions, 36 false-negative patients who satisfied the above-described criteria of the false-negative patients (negative group) and 202 true-positive patients (positive group) were served as subjects for the present investigation. The final diagnosis for all patients with colorectal cancer was made after surgical resection or endoscopic polypectomy.

False-negative cases were identified by the results of colorectal cancer screening the following year (annual screening), consulting the cancer registration records, and information on the morbidity and mortality of colorectal cancer provided by medical facilities.

The principles and procedures of the immunochemical slide Imdia-HemSp (a reversed passive hemagglutination test), which was used in the present investigation, are outlined as follows. The test subjects are instructed first to make a thin fecal smear on the test filter paper. Disks of the specimen from the feces-smeard slides are placed in round wells in a microtiter plate. A diluent is added to extract the specimens from the disks. A portion of the extract is removed and deluted serially in the next three wells of the plate. Erythrocytes coated with anti-human-hemoglobin antibody are added to the last wells, and the mixture is incubated at room temperature for 30 minutes. Samples showing agglutination at a dilution of 1:8 are interpreted as a positive result. The absence of agglutination is interpreted as a negative. The procedures of this test are uncomplicated and the cost per slide for each test is approximately $4.00.

Statistical analysis was performed using McNemar's test, and P value

of less than 5% was defined as statistically significant.

Results

1. Tumor site

In the negative group, the lesion sites included the rectum in 20 (56%), sigmoid colon in 12 (33%), descending colon in 1 (3%), transverse colon in 1 (3%), ascending colon in 2 (6%) and cecum in 0 (0.0%) cases, whereas in the positive group, the lesion sites included the rectum in 60 (30%), sigmoid colon in 80 (40%), descending colon in 14 (7%), transverse colon in 16 (8%), ascending colon in 26 (13%) and cecum in 6 (3%) cases. Accordingly, the prevalence of rectal cancer was significantly higher in the negative group than in the positive group ($p<0.05$) (Table 1).

2. Tumor size

Colorectal cancers were divided into those with a size smaller than2cm and those larger than 2cm. In the negative group, 19 (53%) lesions were smaller than 2cm and 17 (47%) lesions were larger than 2cm. In the positive group, 108 (54%) lesions were smaller than 2cm and 94 (47%) lesions were larger than 2cm, suggesting the absence of any significant difference in lesion size between the two groups (Table 2).

3. Dukes' stage

There were 18 (50%) Dukes C cancers in the negative group and 93 (46%) in the positive group, suggesting that the frequency of Dukes A cancers was highest in both groups. There was no significant difference in the combined frequency of Dukes B and C cases found between the two groups (Table 3).

4. Histological type

In both groups, well-differentiated adenocarcinoma accounted for more than half the cases, followed by moderately-differentiated and poorly-differentiated adenocarcinomas in this order. There was no significant difference in the distribution of histological type found between the two groups (Table 4).

Discussion

There are several factors that may lead to a negative result on fecal occult blood test in colorectal cancer, such as lower sensitivity of screening test, poor preservation of fecal specimens, no bleeding or intermittent bleeding from cancer, inappropriate sampling of stool, and intra-intestinal degeneration of human hemoglobin. In the present investigation, a sensitive test kit was used, in which the cut-off value is adjusted to nearly the upper limit of physiological bleeding (6), and also an experienced laboratory technician exercised special care during the treatment of specimens when conducting the test. Therefore, in the present study, factors such as lower sensitivity and poor storage of sample can be excluded from the possible causes of negative results.

Accordingly, the three factors including no bleeding or intermittent bleeding, inappropriate sampling, and intra-intestinal degeneration of hemoglobin are likely to have constituted the major reasons for negative results on fecal occult blood test. Of these factors, it has been pointed out that intermittent bleeding is the major possible cause of a negative result of fecal occult blood in the study of small colorectal neoplasia (7,8) As to

inappropriate sampling, it has been suggested that in rectal neoplasia in particular, blood can only adhere to part of a stool. Therefore, it was emphasized that a negative test result may be obtained, depending on the site of stool sampling (9).

Moreover, it has been suggested that degeneration of hemoglobin is the possible cause of a negative test result in the study of neoplastic lesions in right side colon (10,11). This reasoning was based on the findings that in right side colon neoplasia, even if the bleeding from lesions exists, hemoglobin peroxidase activity and antigen determinant radical were destroyed by intestinal flora and enteric juice.

In the present comparative study, the prevalence of rectal cancer was higher in the negative group than in the positive group, and no significant difference was observed in the frequencies of right side colon between the two groups. These results suggest that inappropriate stool sampling may lead to a false-negative result on the immunochemical fecal occult blood test, especially in rectal cancer, and that special attention should be paid to the method of stool collection in order to improve sensitivity of this test for rectal cancer. So far, a recommendation for identifying rectal neoplasia that stool should be sampled through an area longer than 5cm, and be taken from 3 regions by brushing (12).

The analysis of clinico-pathological features of false-negative patients is helpful to determine the characteristics of interval cancer. However, the interval cancer patients consist of patients who were discovered in the time course between the initial screening and rescreening. In a strict sense, therefore, the interval cancer is not consistent with the false-negative

cancer defined in the present study. The incidence of interval cancer reported in Western countries ranges widely between 20-55% (13-15). According to the report of screening program for colorectal cancer in Japan, based on the above-mentioned definition of false-negative cancer, the incidence of false-negative cancer is 14% (16).

In conclusion, as to the diagnosis for colorectal cancer, immunochemical fecal occult blood testing has some disadvantages, especially its limited diagnostic accuracy for rectal cancer. In the future, more effective use of this test will depend on a full understanding of its usefulness and limitations.

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Table 1 Comparison of tumor site between colorectal cancer cases with a false-negative fecal occult blood test versus those with a true-positive result in a screening-program based nested case-control study

Tumor site	Results of immunochemical fecal occult blood test		
	Negative (%)	Positive (%)	Total
Rectum	20 (55.6)*	60 (29.7)	80 (33.6)
Sigmoid colon	12 (33.3)	80 (39.6)	92 (38.7)
Descending colon	1 (2.8)	14 (6.9)	15 (6.3)
Transverse colon	1 (2.8)	16 (7.9)	17 (7.1)
Ascending colon	2 (5.6)	26 (12.9)	28 (11.8)
Cecum	0 (0.0)	6 (3.0)	6 (2.5)
Total	36 (100.0)	202 (100.0)	238 (100.0)

* $P<0.05$ for difference between negative and positive groups

Table 2 Comparison of tumor size between colorectal cancer cases with a false-negative fecal occult blood test versus those with a true-positive result in a screening-program based nested case-control study

Tumor size (mm)	Results of immunochemical fecal occult blood test		
	Negative (%)	Positive (%)	Total
<20	19 (52.7)	108 (53.5)	127 (53.4)
20≦	17 (47.2)	94 (46.5)	111 (46.6)
Total	36 (100.0)	202 (100.0)	238 (100.0)

Table 3 Comparison of Dukes' stage between colorectal cancer cases with a false-negative fecal occult blood test versus those with a true-positive result in a screening-program based nested case-control study

Dukes' stage	Results of immunochemical fecal occult blood test		
	Negative (%)	Positive (%)	Total
Dukes A	18 (50.0)	93 (46.0)	111 (46.7)
Dukes B	11 (30.5)	58 (28.7)	69 (29.0)
Dukes C	7 (19.4)	51 (25.2)	58 (24.4)
Total	40 (100.0)	202 (100.0)	238 (100.0)

Table 4 Comparison of histological type between colorectal cancer cases with a false-negative fecal occult blood test versus those with a true-positive result in a screening-program based nested case-control study

Histological type	Results of immunochemical fecal occult blood test		
	Negative (%)	Positive (%)	Total
Well*	24 (66.7)	127 (62.9)	151 (63.4)
Moderately**	9 (25.0)	56 (27.7)	65 (27.3)
Poorly***	3 (8.3)	19 (9.4)	22 (9.2)
Total	36 (100.0)	202 (100.0)	238 (100.0)

* Well-differentiated adenocarcinoma

** Moderately-differentiated adenocarcinoma

*** Poorly-differentiated adenocarcinoma