Gastrointestinal Tract Cancer Screening Using Fecal Carcinoembryonic Antigen

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Abstract. There is a great need to detect gastrointestinal tract cancer at an early stage. It is well known that most carcinoma tissues of the gastrointestinal tract contain carcinoembryonic antigen (CEA). Stools are a rich source of cells derived from the gastrointestinal tract. We analyzed total fecal CEA in 60 gastrointestinal tract cancer patients, 20 benign gastrointestinal disorder patients, and 240 normal controls, using a simple, reliable method. We compared the sensitivity and specificity of fecal CEA with those of serum CEA and fecal occult blood test (FOBT). The level of fecal CEA in gastrointestinal tract cancer was much higher than controls (44.1±70.1 ng/mg stool vs 3.7±3.5 ng/mg stool, p <0.001) and was not increased in benign gastrointestinal disorders (4.5±8.2 ng/mg stool). Fecal CEA level was >10 ng/mg stool in 22 of 32 samples (69%) from stomach cancer patients and 24 of 28 samples (86%) from colorectal cancer patients. The sensitivity of serum CEA (>5 ng/ml) was 19% in stomach cancer and 39% in colorectal cancer, whereas the sensitivity of FOBT was 13% in stomach cancer and 21% in colorectal cancer. The specificity of fecal CEA was 90% in benign gastrointestinal tract disorders and 93% in normal controls. This specificity was similar to those of serum CEA and FOBT. In conclusion, fecal CEA measurement is superior to serum CEA or FOBT for detection of gastrointestinal tract cancer. Fecal CEA may become the screening test of choice for gastrointestinal tract cancer. (received 20 May 2002; accepted 25 August 2002)

Keywords: gastric cancer, colorectal cancer, carcinoembryonic antigen, fecal analysis, fecal occult blood

Introduction

In 2002, Sandler et al [1] reported that in the USA there are 130,000 cases of colorectal cancer, causing an estimated 55,000 deaths annually. Diagnosis and treatment of colorectal cancer in the USA costs approximately $4.8 billion annually. There is need for chemoprevention and early detection strategies to reduce the morbidity and mortality of colorectal cancer [2]. There is presently great interest in chemoprevention with non-steroidal anti-inflammatory drugs (NSAIDs)[3,4]. However, non-selective NSAIDs and even selective COX-2 inhibitors cause side effects and the risks of long-term treatment must be considered carefully [5,6].

Early detection of colorectal cancer is therefore most important. The fecal occult blood test (FOBT) is presently the only laboratory test for population screening for colorectal carcinomas [7,8]. The effectiveness of FOBT to reduce mortality from colorectal carcinoma is still controversial [7,9] because of the high incidence of false positive and false negative results. However, recent colorectal cancer screening studies indicate that use of FOBT can detect about one third of colon cancers and costs only $2,500 per life-year saved [10]. Colonoscopy is effective in detecting colorectal lesions, but it is expensive and invasive as a screening tool [11,12]. Tomography detects colorectal lesions larger than 6
mm in diameter with the same sensitivity as colonoscopy, but it is also unsuitable as a screening method for the same reasons: expense and invasive-ness [13].

There is also a great need to detect and diagnose upper G-I tract cancer at an early stage, before the patient has developed an inoperable tumor or metastases. FOBT is not useful for detecting cancers of the upper G-I tract, such as gastric carcinoma, because the globin in hemoglobin is digested. The serum CEA level is also not recommended for screening of gastric carcinoma. There is no suitable laboratory test and the only available methods to screen for gastric carcinoma are endoscopic and radiologic examinations, both of which are invasive and expensive.

From immunohistological and immuno-cytological studies, it is well known that most carcinomas of the G-I tract contain tumor markers such as carcinoembryonic antigen (CEA)[14]. CEA consists of a large family of related cell surface glycoproteins; it is a stable protein marker for colorectal, gastrointestinal, lung, and breast carcinomas [15]. However, because the serum CEA level is elevated in several benign diseases and is increased in only a small proportion of colorectal cancer patients, serum CEA testing should not be used to screen for colorectal cancer [16,17].

Mutation of the p53 gene is the most common genetic alteration in colorectal cancers; mutations are present in 71% of colorectal carcinomas. A novel examination for alterations of 3 target genes, including TP53, in stool samples can detect over 70% of colorectal cancers [18]. The mutant p53 protein is accumulated in nuclei [19]. Immunohistochemical expression of p53 protein is demonstrable in colon cancers at incidences that range from 70% to 76% [20].

If adequate numbers of cancer cells or their products are mixed in the stool, the amounts of fecal CEA and fecal p53 protein should be higher in patients with G-I tract cancer than in normal controls. Feces is an important excretory product that is generated with predictable regularity. Since stools are a rich source of cells derived from the gastrointestinal tract [20], they are being used for molecular biologic study [21,22]. There should be much oncoprotein derived from intact tumor cells or tumor cell debris in the stools of patients with G-I tract cancers. Theoretically, fecal CEA testing should be suitable for early detection of gastrointestinal cancers, because most carcinomas of the G-I tract contain CEA. In the present paper, we studied fecal CEA and fecal p53 protein levels and we delineate the potential use of fecal CEA as a screening test for G-I tract cancer.

**Materials and Methods**

**Patients.** We studied 60 patients who suffered from gastrointestinal tract cancer (32 stomach cancer, 28 colorectal cancer). The diagnoses of the patients were summarized in Table 1. The 33 male and 27 female patients ranged from 37 to 81 yr old. Stool specimens were collected within 1 wk after the histologic diagnoses were made. Stool specimens from 20 patients with benign gastrointestinal tract disorders (15 chronic inflammation of gastric mucosa, 3 chronic inflammation of colonic mucosa, 2 adenoma of the colon) were also analyzed. Stool specimens from 240 normal healthy controls (12 male and 8 female children, and 118 male and 102 female adults who ranged from 32 to 72 yr old) were analyzed as normal controls. None of them showed abnormalities on physical examination. In 220 normal adult controls, no abnormalities were found in gastric endoscopy carried out for screening. They also had normal serum levels of alpha-fetoprotein, cytokeratin-19 fragment, carbohydrate antigen 19-9, and squamous cell carcinoma-related antigen. All of the patients and all of the normal controls gave their informed consent to participate in this study. Stool samples from 7 volunteers (the authors) were collected between 48 to 72 hr after eating food containing 200 g of swine intestine and 200 g of bovine blood. These subjects were included in the normal controls. Venous blood samples were collected to study serum CEA levels.

**Determination of occult blood in stool (FOBT).** Occult blood in stool was assayed within 2 hr after collection by a latex agglutination inhibition test for human hemoglobin in stool (Iatron Lab, Tokyo, Japan). Results were reported as positive or negative.
Quantitative analysis of CEA in stool and blood. About 80 mg of each stool specimen (84±49 mg) was added to 800 µl of phosphate buffered saline (PBS, pH 7.4), and stored at -80°C until analysis. After thawing in a 37°C water bath, the contents were mixed by vigorous shaking using a vortex mixer. After the freezing and thawing procedure was repeated once more, the mixtures were filtered through a polyvinyl alcohol sponge filter. The repeated freezing and thawing process breaks intact cells in the stool and releases the intracellular CEA. The amounts of CEA in the filtrates and in serum samples were measured using an automated immunoassay system (Elecsys 2010, Roche Diagnostics, Germany), according to the manufacturer's instructions.

Quantitative analysis of p53 protein in stool. The stool filtrates used for CEA determinations were also used for quantitative analysis of p53 protein. The quantitative assays of p53 protein by an ELISA test (p53 pan ELISA kit, Roche Diagnostics, Germany) were performed in duplicate, according to the manufacturer's instructions.

Statistics. All data was analyzed using SPSS software. Statistical significance was evaluated by the Mann-Whitney U test. The Pearson correlation coefficient was used to measure correlation between the levels of CEA in serum and feces.

Results

Fecal occult blood test (FOBT). Ten of 60 samples (16.7%) from gastrointestinal tract cancer patients showed positive FOBT. Four of 32 samples (12.5%) from stomach cancer patients and 6 of 28 samples (21.4%) from colorectal cancer patients showed positive FOBT. Two of 20 samples (10.0%) from patients with benign gastrointestinal tract disorders and 10 of 240 samples (4.2%) from the normal controls yielded positive FOBT (Table 1). All 7 samples of feces collected after ingestion of food containing animal blood yielded negative FOBT.

Quantitative analysis of serum and fecal CEA. The amount of fecal CEA in patients with G-I tract cancer was much higher than in the controls (44.1±70.1 ng/mg stool vs 3.7±3.5 ng/mg stool, p <0.001). The amount of fecal CEA in gastric cancer patients was much higher than in the controls (42.5±57.4 ng/mg stool vs 3.7±3.5 ng/mg stool, p <0.001); there was no significant difference between patients with early gastric cancer and those with invasive gastric cancer (42.0±89.6 ng/mg stool vs 42.9±38.8 ng/mg stool). The amount of fecal CEA was also much higher in patients with colorectal cancer (45.2±63.8 ng/mg stool). The amount of fecal CEA was not increased in patients with benign gastrointestinal disorders (4.5±8.2 ng/mg stool) (Table 2). Serum CEA levels in patients with gastrointestinal tract cancer (6.43±11.85 ng/ml) were significantly higher than those of normal controls (1.14±1.01 ng/ml, p <0.05).

Twenty-two of 32 samples (69%) from stomach cancer patients and 24 of 28 samples (86%) from colorectal cancer patients showed fecal CEA levels >10 ng/mg stool. Serum CEA levels >5 ng/ml (reference range of our laboratory is 0 to 5 ng/ml) were found in 6 of 32 stomach cancer patients (19%) and in 11 of 28 colorectal cancer patients (39%). All of the 17 patients with serum CEA level >5 ng/ml had fecal CEA levels >10 ng/mg stool. Only 2 of 20 patients (10%) with benign gastrointestinal tract disorders (chronic inflammation of gastric mucosa) and 17 of 240 normal controls (7%) had fecal CEA levels >10 ng/mg stool. All stool samples that were collected after control subjects ate food containing animal intestine and blood showed fecal CEA levels <10 ng/mg stool. Serum CEA level was >5 ng/ml in 1 of 20 patients (5%) with benign gastrointestinal disorders and in 8 of 240 normal controls (3%) (Table 1).

There was no significant correlation between serum CEA and fecal CEA levels (p = 0.15).

Quantitative analysis of p53 protein in stool. The lower detection limit of the p53 protein assay was 0.09 pg/mg stool. Twelve of the entire group of 320 subjects (4%) revealed elevated amounts of fecal p53 protein. Among them, there were 9 normal controls, 2 colorectal cancer patients, and 1 with chronic colitis. No significant differences in fecal p53 protein levels were noted among the groups of subjects.
Table 1. Sensitivity and specificity of each test in healthy control subjects and in patients with stomach cancer, colorectal cancer, and benign G-I disorders. The discrimination levels for positive and negative results were: 5 ng/ml for serum CEA, 10 ng/mg feces for fecal CEA, and 0.09 pg/mg feces for fecal p53 protein.

<table>
<thead>
<tr>
<th>Subject category</th>
<th>No. of subjects</th>
<th>Fecal occult blood test</th>
<th>Serum CEA (ng/ml)</th>
<th>Fecal CEA (ng/mg stool)</th>
<th>Fecal p53 (pg/mg stool)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>neg</td>
<td>pos</td>
<td>neg (≤5)</td>
<td>pos (&gt;5)</td>
<td>neg (≤10)</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>32</td>
<td>28</td>
<td>4</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(87.5%)</td>
<td>(12.5%)</td>
<td>(81.3%)</td>
<td>(18.7%)</td>
<td>(31.3%)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>28</td>
<td>22</td>
<td>6</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>(78.6%)</td>
<td>(21.4%)</td>
<td>(60.7%)</td>
<td>(39.3%)</td>
<td>(14.3%)</td>
</tr>
<tr>
<td>Benign G-I disorders</td>
<td>20</td>
<td>18</td>
<td>2</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(90.0%)</td>
<td>(10.0%)</td>
<td>(95.0%)</td>
<td>(5.0%)</td>
<td>(90.0%)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>240</td>
<td>230</td>
<td>10</td>
<td>232</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(95.8%)</td>
<td>(4.2%)</td>
<td>(96.7%)</td>
<td>(3.3%)</td>
<td>(93.9%)</td>
</tr>
<tr>
<td>Total subjects</td>
<td>320</td>
<td>298</td>
<td>22</td>
<td>294</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>(93.1%)</td>
<td>(6.9%)</td>
<td>(91.9%)</td>
<td>(8.1%)</td>
<td>(79.7%)</td>
</tr>
</tbody>
</table>

Table 2. Carcinoembryonic (CEA) levels in serum and fecal samples from healthy control subjects and from patients with G-I tract diseases, categorized according to their diagnoses.

<table>
<thead>
<tr>
<th>Subject category</th>
<th>Subgroup</th>
<th>No. of cases</th>
<th>CEA in feces (ng/mg stool)</th>
<th>CEA in serum (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach cancer</td>
<td>Early gastric cancer</td>
<td>13</td>
<td>42.0 ± 89.6</td>
<td>3.13 ± 2.51</td>
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<tr>
<td></td>
<td>Invasive gastric cancer</td>
<td>19</td>
<td>42.9 ± 38.8</td>
<td>5.09 ± 8.57</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td></td>
<td>28</td>
<td>45.2 ± 63.8</td>
<td>8.87 ± 13.28</td>
</tr>
<tr>
<td>Benign G-I disorders</td>
<td></td>
<td>20</td>
<td>4.5 ± 8.2</td>
<td>1.66 ± 2.03</td>
</tr>
<tr>
<td>Healthy controls</td>
<td></td>
<td>240</td>
<td>3.7 ± 3.5</td>
<td>1.14 ± 1.01</td>
</tr>
</tbody>
</table>
Discussion

About two-thirds of colorectal cancers bleed in the course of a week [23]. But the bleeding tends to be intermittent, and blood is distributed unevenly in the stool. Therefore, the sensitivity of FOBT increases with the number of samples per stool and the number of stools sampled [24]. A study of positive FOBT showed that roughly one-third are caused by bleeding from the anal canal, one-third from colorectal inflammation, and one-third from cancer and polyps [25]. The positive predictive value of FOBT in two controlled trials was 10% for carcinoma and 30% for adenomas for the initial screening test [26,27]. Therefore, fecal blood tests appear to be poor screening procedure for colorectal neoplasia[28]. In this study, only 10 of 60 samples from gastrointestinal tract cancer patients (17%) gave positive results of FOBT. Ten of 240 samples from normal controls (4%) showed positive reactions to FOBT. This low sensitivity may be partially due to a small number of sampled specimens per patients.

All other studies, including fecal CEA assays, were performed on the same stool specimens and the random sampling errors were the same. FOBT is not specific for cancer or blood itself, since non-neoplastic lesions, such as gum disease, gastritis, peptic ulcer disease, and hemorrhoids can also cause gastrointestinal bleeding, and since many substances other than blood can cause positive results for FOBT. Bleeding from upper G-I tract, such as esophagus or stomach, cannot be detected by FOBT owing to degradation of globin by digestive enzymes. In this study, the sensitivity of FOBT was lower in gastric cancer than in colorectal cancer (13% vs 21%). Assays of the stools that were collected after ingestion of animal intestine and blood did not show positive FOBT. This indicates that the FOBT used in this study is specific for human blood.

Fecal CEA level has not previously been studied using a simple enzyme-immunoassay method. In the present study, repeated freezing and thawing of stool in the buffer was used to extract the fecal CEA. It is easy to measure CEA in the extract using an automated enzyme-immunoassay instrument. The antibody used in the Roche Elycys system reacts with CEA and with meconium antigen, which is a non-specific cross-reacting antigen (NCA) [32].

Normal feces contain both CEA and NCA antigens [33]. Most colorectal cancer cells synthesize NCA more actively than normal colon mucosa [34]. If we can discriminate cancer patients from normal persons, it is unimportant which antigens the antibodies bind. The amount of fecal CEA in patients with gastrointestinal tract cancer was much higher than in controls (44.1±70.1 ng/mg stool vs 3.7±3.5 ng/mg stool). The amount of fecal CEA was not increased in benign gastrointestinal disorders (4.5±8.2 ng/mg stool).

The sensitivity of the fecal CEA test to detect gastrointestinal tract cancer was much higher than the FOBT or serum CEA tests. Forty-six of 60 stool specimens from gastrointestinal tract cancer patients (77%) revealed CEA >10 ng/mg stool. Notably, 24 of 28 stool specimens from colorectal cancer patients (86%) yielded CEA >10 ng/mg stool. This sensitivity is very high. And fecal CEA was also increased in 69% of stomach cancer patients, including those with early gastric cancers. There is currently no tumor marker available to screen for stomach cancer. Therefore, periodic measurement of fecal CEA could be a valuable screening tool for stomach cancer in endemic areas like Korea and Japan.

The specificity of fecal CEA was also good >90%). However, serum CEA levels >5 ng/ml (upper limit of reference value of our laboratory) were found only in 17 of 60 gastrointestinal tract cancer patients (28%) and the FOBT was positive in only 10 of 60 gastrointestinal tract cancer patients (17%). It is surprising that the sensitivity of measuring fecal CEA
in only one stool sample per patient is much higher than the conventional screening tests. If consecutive stool specimens were analyzed, the sensitivity would be higher. Until now, FOBT has been the only laboratory test used in population screening for colorectal carcinoma [7,8]. The effectiveness of FOBT to reduce mortality from colorectal carcinoma is controversial because of high false-positive and false-negative results [8,9,24]. In the present study, fecal CEA measurement was clearly superior to FOBT as a screening test for G-I tract cancer.

Stool p53 protein levels were not significantly increased in the gastrointestinal tract cancer patients. Only 12 of the entire group of 320 subjects (4%) revealed fecal p53 protein levels that exceeded the detection limit. Among them, there were 9 normal controls, 2 colorectal cancer patients, and 1 patient with a benign gastrointestinal disorder (chronic colitis). In normal persons, about $3 \times 10^6$ cells can be isolated from a gm of fresh stool [20]. We analyzed about 80 mg of stool/sample; the sample contained $<3 \times 10^5$ cells, and the p53 protein derived from this number of cells could be too small to detect. Since p53 protein is located in the nucleus, it is possible that p53 protein is hard to extract from nuclei of the cells and/or it decomposes rapidly in the stool.

In conclusion, fecal CEA assay is superior to serum CEA or FOBT for detecting G-I tract cancer. Based on our experience, we propose that fecal CEA measurement be used as to screen patients for gastrointestinal tract cancer.

References


