Carcinoembryonic Antigen (CEA) in Gastric Juice or Feces as an Aid in the Diagnosis of Gastrointestinal Cancer

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Carcinoembryonic antigen (CEA) levels in the feces and serum were evaluated in 22 colorectal cancer patients and 20 healthy volunteers; in CEA levels gastric juice and serum were also evaluated in 28 gastric cancer patients and 14 peptic ulcer patients. Fecal CEA was found in all of 22 colorectal cancer patients as well as in the 20 healthy volunteers. Elevated fecal CEA levels were observed in the colorectal cancer patients, as compared to the healthy volunteers. The feces of 15 of the 22 colorectal cancer patients contained CEA at concentrations higher than the mean value plus twice the standard deviation of the healthy volunteers. The fecal CEA levels did not correlate directly either with Dukes' stage or serum CEA levels. CEA in gastric juice was elevated significantly in 26 of gastric cancer patients, with the exception of two patients with early gastric cancer. On the other hand, serum CEA was elevated in only nine of the 28 gastric cancer patients. These results point out the distinct value of assaying CEA in the feces or gastric juice as an aid in the diagnosis of colorectal or gastric cancer.

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W HEN CARCINOEMBRYONIC ANTIGEN (CEA) was discovered in 1965,[8] it was thought to be specifically associated with digestive cancer tissues and fetal tissues. Thereafter, it was found in many other tumors[15] as well as in several normal organs.[15] Circulating CEA levels in serum are frequently raised in nonneoplastic diseases.[12,14,17] These observations about CEA are rather disappointing for diagnosis of digestive cancer. However, CEA levels in blood have proved valuable for monitoring tumor-bearing patients. The limitation of diagnosis by the blood CEA level must be attributed to the extemely low titers of CEA in the blood, more than to any other factor. On the other hand, Livingstone et al.[11] reported that some inoperable colorectal cancer patients with circulating CEA demonstrated, post mortem, quite high titers of CEA in the tumor mass itself; and they suggested that the circulating CEA levels are due to the ability of the neoplasm to release the CEA into the circulation. Furthermore, Sugarbaker[18] reported that four colorectal cancer patients, who had resectable tumors causing large bowel obstruction with colonic dilatation, had very high titers in blood CEA; they then had a prompt fall in serum CEA titers due to the relief of obstruction, yet with the primary tumor

remaining untouched. These observations suggest that CEA is released more into the gut lumen than into the blood stream. For the purpose of alleviating difficulties concerning diagnosis of cancer by blood CEA, the present study of assessing the CEA levels in gastrointestinal fluids or contents, including the almost certain prominent direct scattering from the tumor, was carried out.

Materials and Methods

The fecal samples were collected from 22 patients with colorectal cancer ranging in age from 41 to 74 years with a mean age of 55, and from 20 healthy volunteers ranging in age from 40 to 67 years with a mean age of 51. Of 22 colorectal cancer patients ten were male and 12 were female, and of 20 healthy volunteers 18 were male and two were female.

The gastric juice samples were collected through a gastric tube from 14 patients with gastric or duodenal ulcer ranging in age from 24 to 57 years with a mean age of 35, and from 28 patients with gastric cancer ranging in age from 38 to 69 years with a mean age of 54. Of 14 peptic ulcer patients, ten were male and four were female and among 28 gastric cancer patients 17 were male and 11 were female. The diagnosis of benign gastric ulcer was based firstly on radiographic and gastroscopic evidence, then on possible healing with internal medical treatment, and finally on microscopic examination of resected specimens and/or endoscopic biopsy specimens. The diagnosis of malignant diseases was confirmed by microscopic examination.

Gastric juice free of bile or blood was chosen, as were feces without barium or blood. About 500 mg of feces was put into a ten fold quantity of 0.1 M acetate buffer (pH 5.0), and dissolved entirely on a stirrer. The mixture was centrifuged at 2500 g for ten minutes and the supernatant was analyzed immediately. The gastric juice and blood for serum were centrifuged at 2000 g for ten

minutes and the supernatants were either analyzed immediately or kept frozen until analysis.

The CEA levels in these supernatants were measured by a radioimmunoassay utilizing the "one step sandwich method" by Hirai.[9] A detailed description of this method has been reported previously[7,9] and is here briefly summarized. These supernatants, which were diluted with an equal volume of acetate buffer, were incubated at 85° for ten minutes in a water bath and thereafter were centrifuged for five minutes at 2500 g. After centrifugation, the supernatant was used for the radioimmunoassay. Each 100 μl of the sample and ^{125}I-anti CEA antibody was pipetted on an anti-CEA antibody coated disc in the well of a measuring plate. The plate was shaken on a shaker for 24 hours at room temperature. Unbound ^{125}I-anti CEA antibody was removed from each well and then ^{125}I-radioactivity, which adheres well to each disc under the "sandwich" state, was counted in γ-scintillation counter. Each CEA level was calibrated from the standard curve obtained through CPM of standard CEA solution. This one step sandwich method, which was established by Hirai,[9] replaces the troublesome extraction with perchloric acid and its dialysis by a simple procedure consisting of incubation at 85° for ten minutes. It is reported by Hirai[9] that, irrespective of the difference between CEA extraction by heat treatment at 85° and by perchloric acid, the CEA values obtained with this method and Hoffman-La Roche method correlated quite well. He

further reported that, by this one step sandwich method, one ng of purified CEA gave about 800 cpm, yet over 1000 ng of nonspecific cross-reacting antigen-2 (NCA-2) designated by Burtin et al.[1] barely managed to give the same count. The CEA level in the feces is represented as ng/g in wet weight of the feces and that in gastric juice and serum as ng/ml.

Results

CEA in the Feces

CEA activity was found in the feces of all 20 healthy volunteers and 22 colorectal cancer patients. As shown in Figure 1, the mean and standard deviation of the fecal CEA activity from the volunteers, four patients with Dukes' A tumor, three patients with Dukes' B tumor, nine patients with Dukes' C tumor, and six patients with liver metastasis were 78 ± 42 ng/g, 193 ± 51 ng/g, 178 ± 73 ng/g, 213 ± 90 ng/g, and 267 ± 95 ng/g, respectively. The mean fecal CEA level of the 22 patients with colorectal cancer was significantly higher than that of the healthy volunteers ($p < 0.001$), using the Student's t-tests.[10]

There was no difference in the fecal CEA level among the four groups with Dukes' A, B, C, and liver metastasis. There was no apparent relationship between CEA levels in the feces and those in the blood, with the single exception being the patient group with liver metastasis (Fig. 1).
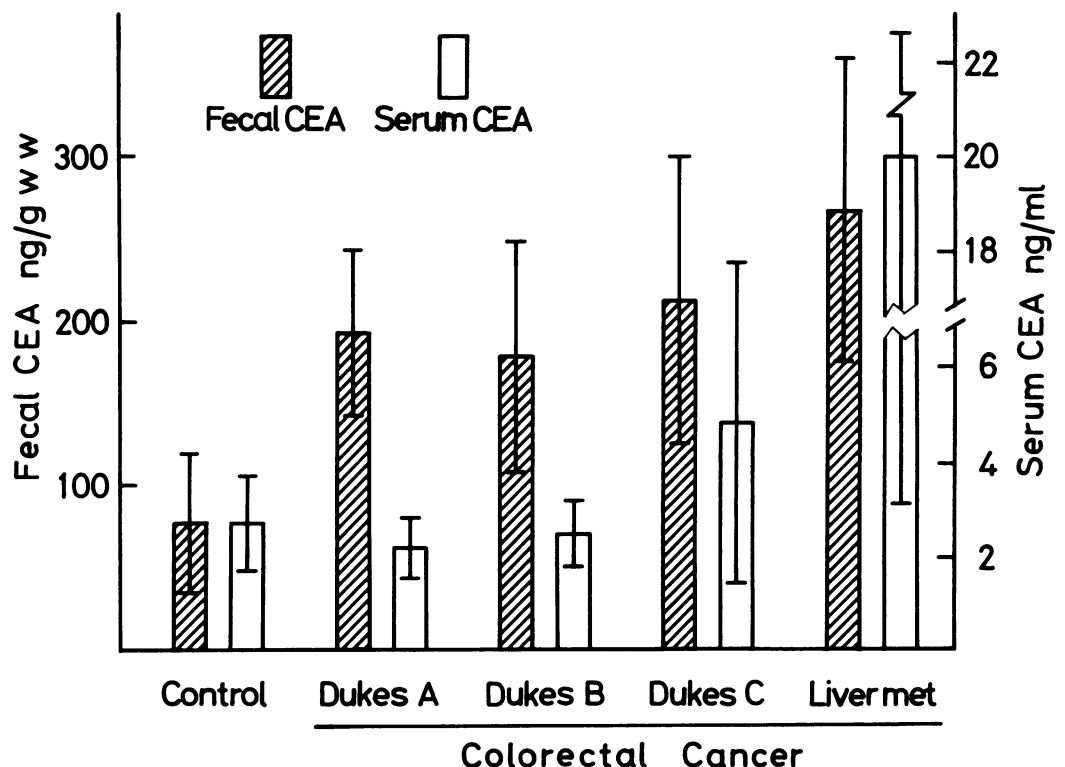

FIG. 1. CEA levels in the feces and serum of 22 patients with colorectal cancer. The values represent mean ±S. D. Control, 20 healthy volunteers. The numbers of patients with Dukes' A tumor, Dukes' B tumor, Dukes' C tumor and liver metastasis are 4, 3, 9, and 6, respectively.
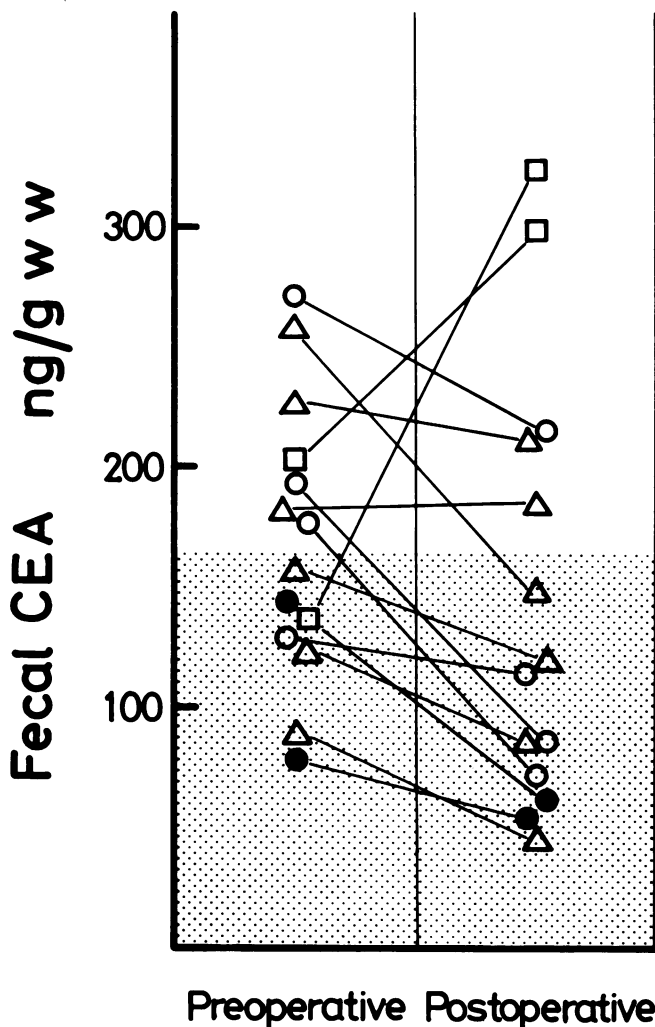
FIG. 2. Comparison of pre- and postoperative fecal CEA levels in patients with colorectal cancer. Dotted area represents the normal range (mean ±2 × S. D.) of fecal CEA levels in controls. O, patients with Dukes' A tumor; ●, patients with Dukes' B tumor; △, patients with Dukes' C tumor; □, patients with liver metastasis.

In 14 patients treated by surgical resection, the fecal CEA levels were determined before and after operation (Fig. 2). In nine of 14 patients, the fecal CEA levels clearly decreased after operation, whereas in two patients with hepatic metastases, the levels had, in fact, increased, although colon tumor resection palliatively had been carried out.

CEA in Gastric Juice

CEA activity in gastric juice was detected in all of the patients with stomach cancer and in ten of 14 peptic ulcer patients. As shown in Figure 3, the mean and standard deviation of CEA levels in gastric juice from 14 peptic ulcer patients, four patients with stomach cancer limited to the mucosa (m), three patients with stomach cancer involving the muscularis mucosa (pm),

four patients with stomach cancer reaching the subserosa (ss), and 17 patients with stomach cancer involving the serosa(s) were 0.43 ± 0.06 ng/ml, 1.7 ± 0.8 ng/ml, 2.6 ± 0.3 ng/ml, 2.3 ± 0.9 ng/ml, and 9.6 ± 3.8 ng/ml, respectively. Of the four patients with stomach cancer limited to the mucosa and the four patients with stomach cancer reaching the subserosa, one patient of the former had a rather low CEA level of 0.5 ng/ml and one of the latter a low 0.7 ng/ml. The mean CEA levels from the patients either with stomach cancer limited to the mucosa or with stomach cancer involving the serosa were significantly higher than those from the peptic ulcer patients (the former: $p < 0.01$, the latter: $p < 0.001$), using the Student's t-tests.[10]

Discussion

Many authors have reported that a high blood CEA level at the preoperative stage usually indicated the existence of advanced cancer and that this level was reduced promptly by tumor removal. Undoubtedly the high blood level originates from the mass production of CEA by the tumor. However, many clinicians are anxiously awaiting the development of some viable means of detecting the very low levels of blood CEA at relatively early stage of cancer. The circulating CEA may be controlled by several factors, including the production of CEA by cancer cells, release from the tumor directly into the blood stream and metabolic degradation in the liver.[12,16] However, it is quite within reason, from Sugarbaker's observation,[18] to suggest that fluids in the alimentary canal must include a large quantity of CEA discharged from the tumor itself, and this considerable portion is not exposed to the degradation process in the liver. The present data indicate that a CEA assay of alimentary canal contents or fluids may be a more reliable system as an aid in the diagnosis than that in serum.

In 1972, Freed and Taylor[6] reported by the use of the gel-immunodiffusion method that in the feces of normal volunteers CEA may be present in small amounts but that in malignant conditions of the bowel the amount increases. Elias et al.[5] also reported large quantities of CEA in the intestinal contents under similar conditions. By contrast, it was reported by Egan et al.[4] that CEA-like material obtained from colon lavages of healthy volunteers is immunologically and chemically very similar to, and perhaps identical with, the CEA of tumor tissue. From these observations, the fecal CEA titers estimated by the present authors involve a total of the fecal CEA originated from colorectal tumor, that originated from normal colorectal mucosal cells, and non-specific cross-reacting antigen-2 (NCA-2) designated by Burtin et al.[1] However, Hirai[9] reported that this one step sandwich method cross-reacts only with NCA-2 in
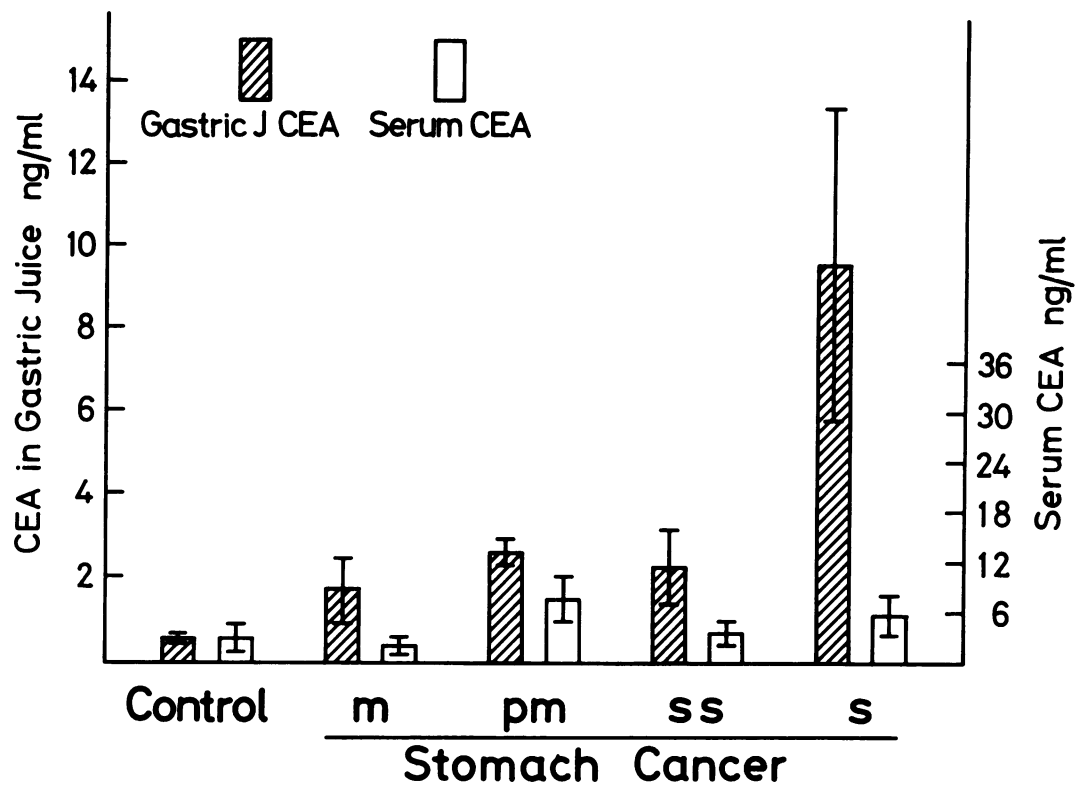
FIG. 3. CEA levels in gastric juice and serum of 28 patients with gastric cancer. The values represent mean ± S.D. Control, 14 patients with peptic ulcer; m, 4 patients with stomach cancer limited to the mucosa; pm, 3 patients with stomach cancer involving the muscularis mucosa; ss, 4 patients with stomach cancer reaching the subserosa; s, 17 patients with stomach cancer involving the serosa.

the proportion of 0.1% to CEA. In addition, the elevated fecal CEA levels in patients with resectable colorectal cancer declined after curative operation to fecal CEA levels near the normal range (Fig. 2). These observations, including the present data, point out a more intimate relationship of colorectal cancer with the fecal CEA titers than with the circulating CEA in blood.

As for CEA levels in gastric juices, Vuento et al.[19] reported that gastric juices of eight normal individuals and five gastric cancer patients contain a CEA-like substance which has antigenic activity according to CEA radioimmunoassay and gives a cross reaction with CEA in immunoelectrophoresis. They further suggest that this CEA-like substance may represent true CEA or be a new member of the CEA glycoprotein family. A similar observation was reported by Deutsch et al.[3] and Molnar et al.[13]

On the other hand, Burtin et al.[2] reported by immunocytological study that the CEA was found either in well differentiated carcinoma of the stomach or in noncancerous gastric glands having undergone an intestinal metaplasia, but the normal gastric mucosal gland was negative for CEA. They further reported that immunofluorescence staining of the gastric mucosa was weaker than that of the colonic mucosa. These observations by Burtin et al.[2] will naturally suggest that the gastric juice also includes a moderate quantity of CEA discharged from the tumor and metaplastic glands.

The present data and the previous observations con-

cerning the CEA-like substance in gastric juice suggest the usefulness of determining its existence for the intended purpose of detecting stomach cancer. CEA in the feces and gastric juice may attain a special place in supplementary diagnosis of gastrointestinal cancer. It is currently very difficult to differentiate with fair certainty between early gastric cancer and gastric ulcer because of the low CEA titers. This fact may limit but not totally negate its value as the complementary diagnosis of gastrointestinal cancer. However, the materials are easily obtainable from all outpatients and the experimental procedures can be performed without any trouble.

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