Value of Tissue Carcinoembryonic Antigen in Patients with Colorectal Carcinoma

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The carcinoembryonic antigen (CEA) was originally reported by Gold and Freedman in 1965, by isolating it from human fetal intestine and adult colon cancer tissue.[1,2] Many studies with CEA have suggested that preoperative serum levels can be used as a prognostic indicator for colorectal carcinoma,[3] as well as a monitor for detection, staging, checking recurrence and determining response of therapy in cancer patients.[4-5] The evaluation of preoperative serum CEA levels in conjunction with the cancer's histologic grade, can be useful in establishing a more accurate staging of the neoplastic disease.[6-7] Elevated serum CEA levels also have been found in association with inflammatory bowel diseases and colorectal polyps,[8-10] in hepatic and pancreatic diseases[11-13] as well as in breast,[14] pancreatic[15] and various other cancers.[16-18]

Practically, the most important use of serum CEA is for early detection of cancer recurrence. Rises in serial CEA levels during follow-up can be the first sign of a relapse before the other clinical signs of the disease can be detected readily by several months, if the CEA is indeed

SUMMARY The tumours of 55 patients with colorectal carcinoma were evaluated for tissue carcinoembryonic antigen (CEA) by immunoperoxidase staining. It was shown that 33/35 patients with increased preoperative serum CEA levels above 5 ng/ml had positive tissue CEA. The other 17/20 patients who had serum CEA levels less than 5 ng/ml could be demonstrated CEA in tissue. The results of tissue CEA were compared with their preoperative serum CEA levels in the pathologic grading, histologic type and staging of cancer. It was found that tissue CEA was more sensitive than serum CEA and was correlated with serum CEA in all respects.

The finding in this study suggests that tissue CEA should be performed along with preoperative serum CEA in all patients suspected of having colorectal carcinoma. The postoperative serum CEA should be determined serially in the patients who have more than 5 ng/ml serum CEA and/or tissue CEA positive although their preoperative serum CEA is less than 5 ng/ml.

present in the original malignant lesion.

This present study was conducted to evaluate the value of tissue CEA in the lesion of colorectal cancer by correlating the results with corresponding preoperative serum CEA levels and the pathologic staging, histologic type and grading of tumor.

MATERIALS AND METHODS

Patients

A study was made of fifty-five patients with primary colorectal adenocarcinoma. Twenty-nine of the patients were male and 26 were female. The ages ranged from 20 to 80 years. All patients underwent surgical treatment to remove neoplastic lesions.

Enzyme-linked immunosorbent assay (ELISA)

The preoperative serum CEA levels were determined in all patients by ELISA test kit (Roche, Bazel, Switzerland) which is based on the sandwich system. Briefly, the patients' sera, CEA standards, negative and postive CEA sera controls were

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incubated in one step with the beads coated with mouse anti-CEA monoclonal antibody and goat anti-CEA peroxidase. After washing, the O-phenylenediamine, which was the substrate, was added. After incubation, the enzyme reaction was stopped by adding HCl solution. The intensity of the color developes was read at 412 nm. The amount of CEA present in the sample was in direct proportion to the intensity of color. A standard curve was constructed by plotting the CEA concentration of the standard samples versus the absorbance. The amount of CEA in each sera was determined directly from the standard curve.

Since a slight elevation of CEA levels up to 5 ng/ml was known to be found at several rates in benign disease, [19-21] CEA levels greater than 5 ng/ml were considered as a cut off level for malignancy in this study.

Histologic examination

Surgically removed specimens were fixed in 10% buffer-formalin and embedded in paraffin. Tumors were classified by degree of differentiation, stage of tumor invasion, nodes metastasis, presence of blood vessels or lymphatic invasion and site of tumor in colon. [22] All sections were stained with Hematoxylin and Eosin (H&E) and elastic Van Gieson's stain. The stage of invasion was determined by a modified Dukes' classification, [23] that is: Stage A lesion was limited to the submucosa, stage B lesion was invaded through the muscularis propria into the serosa without lymph nodes metastasis, stage C lesion involved regional lymph nodes and stage D lesion had distant spread.

Immunoperoxidase (IPx) procedure

A representative block from the primary tumor of each patient was selected. The peroxidase-antiperoxidase (PAP) immune complex was used to determine tissue CEA

staining, using the technique described by Sternberger. [24] Briefly, the sections were incubated with rabbit anti-human CEA (Dako, California) as the primary antibody. Fractions of swine anti-rabbit IgG (Miles Yeda Ltd., Rehovot, Israel) were used to link the rabbit horseradish peroxidase anti-proxidase complex (Miles Yeda Ltd. Rehovot, Israel) to the primary antibody. The 3-3' diaminobenzidine (Sigma Chemical Co., St. Louis) was used as chromogen.

A positive immunoperoxidase reaction consisted of finely granular staining of the cytoplasm or apical border of the malignant cells. Cases were considered negative when staining

was no greater than background staining with normal rabbit serum. Positive control sections consisted of well differentiated colonic carcinoma, known to produce CEA. The negative control using sections incubated with rabbit non-immune serum were also included. IPx staining was recorded as either absent (-) or present (+). Intensity of staining was not found to be useful.

RESULTS

The presence of CEA in neoplastic tissue (Fig. 1) could be demonstrated in 50/55 cases of colorectal adenocarcinoma. However, the intensity of CEA staining in tissue

Table 1. Correlation between serum and tissue CEA

Serum CEA (ng/ml)	Patients (No.)	Tissue CEA + Patients	Tissue CEA + %	Tissue CEA − Patients	Tissue CEA − %
> 5	35	33	94.3	2	5.7
< 5	20	17	85	3	15
Total	55	50	−	5	−

Table 2. Comparison of sensitivity between tissue and serum CEA by using tissue CEA as gold standard

| Pathological findings	Sensitivity Tissue CEA	Sensitivity Serum CEA
Grading of tumor		
well diff. (n=26)	100% (26/26)	65.4% (17/26)
mod. diff. (n=18)	88.8% (16/18)	66.7% (12/18)
poorly diff. (n=11)	72.7% (8/11)	54.5% (6/11)
Histologic type of tumor		
Adenocarcinoma (n=52)	92.3% (48/52)	61.5% (32/52)
Mucinous carcinoma (n=3)	100% (3/3)	66.6% (2/3)
Pathologic staging of tumor		
Stage A (n=1)	−	−
Stage B (n=25)	88% (22/25)	64.0% (16/25)
Stage C (n=29)	93.1% (27/29)	65.5% (19/29)
Stage D (n=0)	−	−
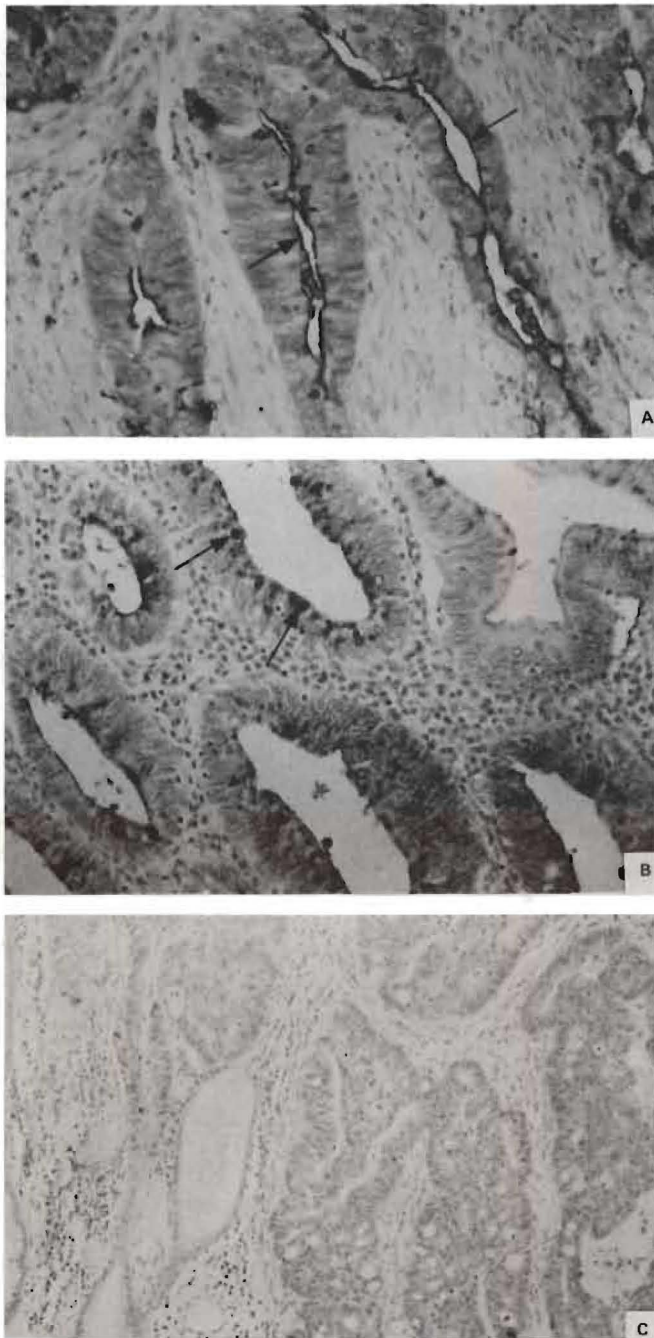
Fig. 1 Positive tissue staining reaction for CEA in colonic carcinoma, mainly in the apical area (A), both apical and cytoplasm (B), and negative control (C).

was identified.

Thirty-three from thirty-five patients (94.3%) with preoperative serum CEA level above 5 ng/ml and 17/20 patients (85%) with preoperative serum CEA level below 5 ng/ml had positive tissue CEA, while 3/55 patients had both negative serum and tissue CEA (Table 1).

When using tissue CEA as gold standard, the sensitivity of tissue CEA was comapred with serum CEA in tumor grading, histologic type and pathological staging of tumor (Table 2). It was revealed that tissue CEA was correlated with serum CEA and more sensitive than serum CEA.

For grading of tumor, the production of CEA in well differentiated adenocarcinoma was more efficient than moderately and poorly differentiated adenocarcinoma respectively. The sensitivity of tissue CEA was greater than serum CEA in all 3 grades of tumor.

For histological type of tumor, 52 patients had adenocarcinoma and 3 patients had mucinous carcinoma. In the adenocarcinoma group, 48 patients (92.3%) had positive tissue CEA while only 32 patients (61.5%) had serum CEA above 5 ng/ml. Three out of three patients (100%) in the mucinous carcinoma group had positive tissue CEA, but only 2 patients had serum CEA above 5 ng/ml. Thus, tissue CEA was more sensitive than serum CEA in this respect.

For pathologic staging of tumor (Dukes' classification), one patient was in stage A, 25 were in stage B, and 29 were in stage C. The sensitivity between tissue and serum CEA was compared only in patients with stage B and stage C. The tissue CEA was more sensitive than serum CEA in both stages. Patients in stage C seemed to produce CEA more efficiently than stage B.

DISCUSSION

Our findings reveal that tissue CEA is more sensitive than but cor-

varied from cell to cell within the same malignant gland and from gland to gland within different part of the same tumor. The site of CEA distribution was not different according to degree of tumor differentiation. Apical surfaces distribution of CEA or intracytoplasmic localization, alone or in combination, were seen in well differentiated tumor as well as moderately or poorly differentiated adenocarcinoma. No stromal staining

related with serum CEA in either pathologic grading or histologic type or staging of colorectal carcinoma. Among 55 patients suffering from this disease, 52 patients (94.5%) had at least one positive parameter, i.e., positive tissue or serum CEA or both, which is an interesting finding. That is the predictive value of CEA is increased when both blood and tissue are examined for CEA by ELISA and IPx respectively.

A majority of the patients (33/55) had increased preoperative serum CEA levels and positive tissue CEA staining reaction. This group of patients should be the most benefit from serial postoperative serum CEA determinations for predicting the progress of disease.

The second group of patients (17/55) had preoperative serum CEA less than 5 ng/ml but positive tissue CEA. The explanations for this group are these: there are many pathophysiologic processes controlling levels of serum CEA which include production of CEA by tumor, release of CEA into surrounding tissue and circulation, metabolic degradation and excretion by the liver, and reabsorption from within the colonic lumen.[25-26] Hence, the level of serum CEA in each individual is different depending on the pathophysiologic processes mentioned above. However, during the disease process, if one has tissue CEA produced by primary tumor, then it would be possible that a certain level of serum CEA from the vast amount of tissue CEA produced by metastatic lesions should be detected. The other possibility is that, the tumor indeed secrete small amount of CEA but the results of serum CEA obtained are false negative due to CEA-antiCEA complex in the serum, because CEA antigen in this complex cannot be seen by monoclonal anti-CEA used in the assay. The serial postoperative serum CEA in this group should be useful for determination of disease progress, since we have found that

the more severe the disease, the more positive tissue and serum CEA were obtained (Table 2).

The third group consisted of two patients (2/55) who had elevated serum CEA (10.6 ng/ml and 109.6 ng/ml respectively) but negative tissue CEA. Both patients had lymph nodes metastasis. Thus, it is possible that CEA was produced by the malignant cells in metastatic lesions rather than primary tumor. This observation also has been reported by other investigators.[27] The postoperative serum CEA determinations in this group should also be useful.

The fourth group are 3 patients (3/55) who had negativity of both serum and tissue CEA. The postoperative serum CEA determinations would not be beneficial to these patients.

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