Acta Med. Nagasaki 31 : 294—302

Clinical Evaluation of Carcinoembryonic Antigen (CEA) in Colorectal Cancer

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Received for publication, July 2, 1986

Carcinoembryonic antigen (CEA) values (Dinabot-kit) were measured in proven 177 patients with colorectal cancer preoperatively, and at routine intervals following operation. The assay was positive in 39.1% with stage I・II, 65.9% with III・IV and 85.7% with V. Elevated CEA levels were noted in those who had infiltation of cancer cells extending through the proper muscle layer. In 79.2% of curative resections CEA levels returned to normal within one month, but the titers remained elevated in 73.3% of palliative resections. Among 26 patients with recurrent disease, 16 had a hepatic metastasis showing a previous or simultaneous CEA rise, whereas 10 had a local recurrence with a slow rise or normal. In addition, a quantitative study of CEA in extracts of 87 tumors was made in order to assess the influential factors on the level of circulating CEA. There was no significant difference between the CEA contents of the tumor and its level of circulating CEA. The level of circulating CEA might be contributable to the spreading cancer cells rather than the tumor containing CEA.

INTRODUCTION

The serial CEA measurement in patients with colorectal cancer is one of the most important and useful means to evaluate effect of staging, prognosis, and monitoring of therapy. On the other hand, since the isolation of CEA from colonic adenocacinoma

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achieved by Gold and Freedman,[1] the cellular localization, tissue distribution, biochemical properties and clinical significance of this antigen have been extensively studied. Although many conventional biochemical or histopathologic studies have been attempted to account for the eleveted serial CEA levels,[1)2)3)]there was little quantitative measurement of tumor CEA in comparison with that of serum CEA.[4)5)]

The present study was undertaken to asses the clinical values of the CEA measurement for colorectal cancer patients and to elucidate the factors responsible for elevation of serum CEA levels.

MATERIALS AND METHODS

One hundred and seventy-seven patients who underwent resection for primary colorectal carcinoma at the First Department of Surgery, Nagasaki University School of Medicine since 1977 were subjected to this study. All have been histopathologically proven cancers of the large bowel and multiple cancers were excluded. There were 85 males and 95 females ranging in age from 22 to 88 years.

Macroscopic classification and microscopic evaluations of the resected specimens were based on the rule of Japanese Research Society for Cancer of the Colon and Rectum.[6)] The blood samples were taken on admission and at one month and every 3 months after surgery. The radioimmunoassays for carcinoembyonic antigen (CEA) were performed by Sandwitch method using Dinabot-RIA Kit, a normal values being less than 2.5 ng/ml.

Extraction of tissue and procedure

The fresh tissue samples were taken from primary tumor, metastatic tumor of liver, regional lymphnode and normal colonic tissues of oral or anal site at least 5 cm distant from the edge of the tumor. Colorectal tumor or metastatic tumor to the liver were dissected free from surrounding normal tissues, and 0.5-1.0g of these tumors were cut into pieces in 3-5ml cold normal saline per gram of tissue and homogenized at 4°C in phosphate -buffered saline of pH 7.2-7.4 with Ultrafurrox homogenizer (HITACHI 20PR-52D). After centrifugation at 1,600*g for 20 min., the supernatant was then tested in the microimmunoassay. The CEA content of tissue was calculated by ng/wet weight of tissue (g).

RESULTS

Preoperative values of CEA

The mean values of serum CEA of 177 patients with colorectal cancers was at a range of 7.40 ± 12.25 ng/ml, and the rate of positive assay was 57 percent. The incidence of detectable serum CEA in relation to the stage of the disease is shown in Fig. 1. A close correlation was observed between serum CEA level and the disease stages. Positive results were recorded in 39.1% of stage I and II, and 69.5% of stage III and IV in which lymphnodes were apparently involved respectively. The highest values per se was found in the patients with stage V that showed liver metastases and/or peritoneal disseminations. Age and sex did not appear to correlate with the CEA titer. On the other hand, in patients potentially cured by surgery, 49.6% of them was positive, while a significantly high positive rate of 81.0% ($P<0.01$) was seen in patients with non-curable resection.

Changes in CEA values after tumor resection

The change of CEA after surgery was influenced on the initial preoperative values and the stage of disease. Fig. 2 showed a variation of CEA levels within several hours after operation. Overall results of 26 patients studied showed a decrease in CEA levels immediately after operation. Of the 17 patients whose preoperative CEA values were higher than 2.5ng/ml, CEA values returned to normal in 5 patients, but in the patients with metastatic lesions the titers failed to returned to normal values. On the other hand, in 79.2% of patients with curative resection CEA levels returned to normal within one month, but the titers remained high in 73.3% of those with palliative resections.

Sequential changes in CEA after curative resection in relation to recurrence

Twenty-six patients with recurrence were sequentially followed with regard to CEA values over a 6-40 months period after curative resection. The types of recurrence were distant metastases in 14 (liver 11, lung 2, thyroid 1) and local recurrence in 10. Preoperative CEA values of patients with recurrence was 4.96 ± 7.9ng/ml (61.5% in positivity), whereas CEA in the patients with non-recurrence was as low as 3.06 ± 3.16ng/ml (37.6% in positivity). The patterns of changes in CEA levels were characteristic of the type and the site of recurrece when recurrence took place.

Fig. 3 illustrated that the CEA levels often rose rapidly prior to clinical evidence of liver metastases as shown in the twelve patients in whom liver metastases after curative

resection developed. All of these patients showed a rapid rise in CEA 5-6 months prior to appearance of clinically detectable recurrence in the liver. In contrast, in patients with local recurrence, the CEA level rose more slowly and often remained within normal limits at the time when clinical recurren verified as shown in Fig. 4. The patterns of CEA elevation differed between patients with local recurrence and liver metastases including peritoneal dissemination. In general, the highest CEA levels was seen in patients with liver meastases.

Correlation of CEA and histopathologic factors

One hundred fourty-nine patients excluding 28 patients with stage V were eligible for this study. The relationship between several histopathologic factors and preoperative serum CEA values was shown in Table 1. The low values of CEA was observed in superficial type (type 0) and protuberant type (type 1), whereas a high level of CEA was observed in ulcerative type (type 1, 2 and 3) according to macroscopic classification. There was a tendency for the tumor of more than 2.0cm in diameter to show a progressive elevation in CEA levels as the tumor mass size increased, although there was no tendency for the tumor of less than 2.0cm in diameter.

All squamous cell carcinomas showed low values of less than 2.5ng/ml. All three

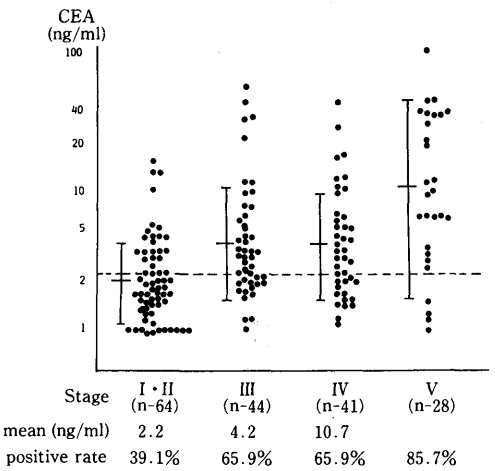

Fig. 1. Serum CEA levels and the stage of disease in colorectal cancer.

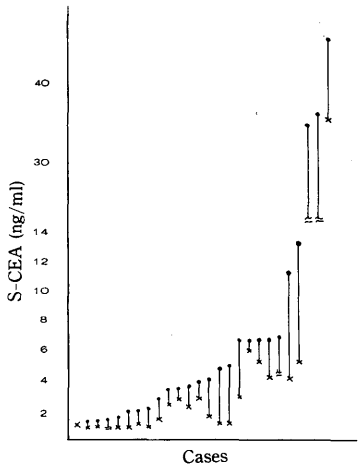

Fig. 2. Variation of CEA levels in several hours after surgery. ●: Preoprative serum CEA levels, ×: Postoperative CEA levels.

mucinous carcinomas accompanying peritoneal dissemination and/or liver metastases showed a remarkable rise with a mean values of 14.1ng/ml. However, there was no close correlation between cell differentiation of adenocarcinoma and serial CEA level. According to depth of tumor invasion, the elevated serial CEA was noted in those with infiltration of cancer cells extending through the proper muscle layer and marked elevation also observed in those with INFγ invasion compared to INFα or INFβ. Although there was no apparent relationship between serial CEA levels and venous or lymphatic vessel invasions, the serial CEA levels tended to be higher in positive vascular invasions than in negative ones.

CEA in tissue extract and serum CEA

The amount of CEA extracted from 87 tumors and various non-cancerous tissues were shown in Fig. 5. Among 87 primary tumors, the amount of CEA ranged from 460 to 29,690 ng/g tissue with a mean values of 7144.0ng/g tissue. Non-cancerous tissues contained one-tenth as low as CEA values as compared to cancerous tissues. As regard to histologic cell differentiation, well and moderately differentiated adenocarcinoma contained greater amounts of CEA than poorly differentiated adenocarcinoma and mucinous carcinoma. High CEA contents was also observed in metastatic lymphnode or metastatic tumor of the liver. No significant correlation between various pathological factors and the amounts of CEA of the tumor was observed. There appeared to be no correlation between CEA contents of the tumor and the corresponding level of CEA in serum obtained preoperatively (Table 2).

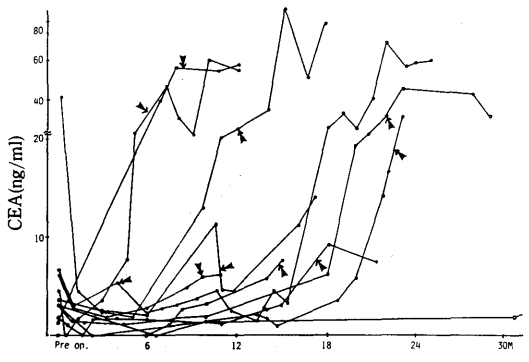

Fig. 3. Variation of CEA levels in patients with liver metastases.

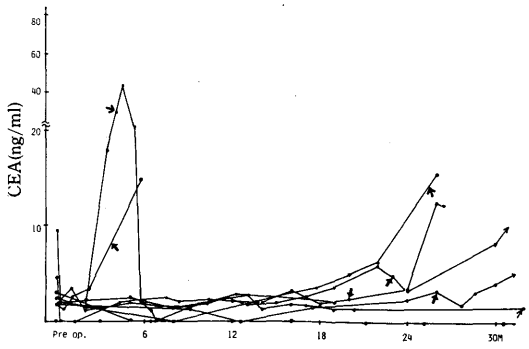

Fig. 4. Variation of CEA levels patients with local recurrence.

Table 1. Correlation between pathological findings and serum CEA levels.

	Serum CEA (ng/ml)	positive rate (%)		serum CEA (ng/ml)	positive rate (%)
gross type			cancer invasion		
0	1.56±1.55	30.0	m sm	1.48±1.52	25.0
1	2.10±1.45	30.0	pm	1.55±1.61	15.4
2	3.16±2.34	54.9	ss(a_1)	3.31±2.26	53.6
3	4.79±2.65	65.2	s(a)(+)	4.27±2.60	70.4
4	6.64±6.98	66.7	v-factor		
p(+)H(+)	10.47±3.39	88.5	V(+)	3.44±2.49	56.0
size of tumor			V(−)	3.16±1.26	51.3
2 cm	1.44±1.49	25	ly-factor		
4	2.88±2.21	42.3	ly(+)	5.48±8.27	56.2
6	2.82±2.27	55.1	ly(−)	2.98±2.87	31.0
8	3.64±2.31	60.0	INF		
8cm～	4.30±2.90	66.7	α	2.71±2.84	40.0
histological type			β	2.95±2.26	50.0
well diff,ad-ca	2.29±2.51	50.0	γ	5.31±2.49	83.3
moderately	3.11±2.26	56.1			
poorly	3.43±2.27	50.0			

DISCUSSION

CEA was first defined as a systemic-specific cancer antigen associated with tumors of the digestive tract by GOLD and FREEDMAN.[1] This cancer specificity has subsequently been questioned by several workers, and recently it has been regarded as a tumor-associated antigen.[4][5] However, in spite of denying the tumor and organ specificities of CEA, CEA measurement seems to be the most reliable in comparison with various markers for colorectal cancer. It has been reported in Japan that positive rate of serum CEA for colorcctal cancer ranged from 35.4% to 69.9%.[7]

In the present study, preoperative CEA level coincided well to the stages of disease according to classification of Japanese Research Society for Cancer of the Colon and Rectum and/or Dukes' classification. The assay also showed a high level in patients with positive lymphnode than those with negative ones, but there was no correlation between the location of positive nodes among stage III and IV. It seems that serum level of CEA is affected by the amount of metastatic nodes rather than the numbers, because n-number described by Japanese rule is named with the degree of a distance from the primary tumor.[6] These findings indicate that the CEA test is insensitive to making diagnosis of colorectal cancer at an early stage. However, there is little doubt that abnormal serum level of CEA is not infrequently found in patients with widely spreading cancer. The CEA

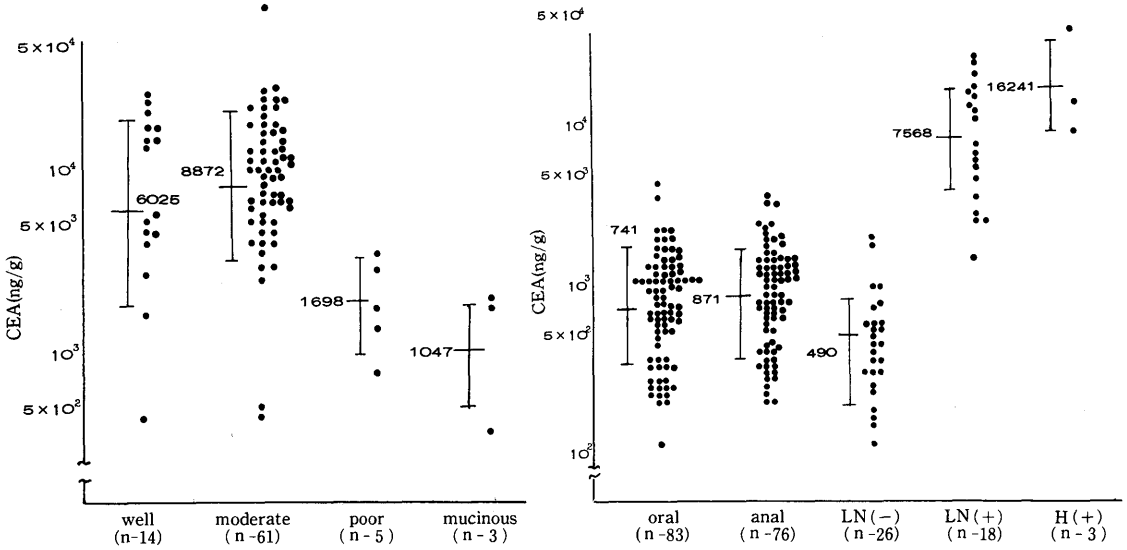

Fig. 5. CEA content in cancerous and non-cancerous tissues of colorectal cancer. LN(−): no metastatic lymphnode, LN(+): metastatic lymphnode, H(+): metastatic tumor of liver.

Table 2. Correlation between serum CEA and CEA content of cancerous tissue in colorectal cancer.

stage	n	tissue CEA (ng/g)	serum CEA (ng/ml)	Positive rate (%)
I II	28	6950.2±4012.6	2.44± 1.09	25.7
III	22	7943.3±4867.2	11.95±14.92	63.6
IV	21	7481.7±5115.1	4.77± 4.17	71.4
V	16	5368.8±3777.0	14.92±14.38	87.5

assay would seems to be most available for assessment of prognosis and in early detection of recurrent disease.

GOLD and FREEDMAN first reported that the CEA would revert to negative following complete tumor resection.[1] The authers have comfirmed that serum CEA level decreased within several hours following curative resection as well as palliative resection of the tumor mass, but not to normal. A decrease in CEA following palliative resection correlated with the remained tumor mass. Therefore, one should take it into consideration that if the CEA levels remain high after surgery, curative resection can not be achieved. Preoperative CEA values were higher in patients with recurrence rather than in those whithout. This finding suggests that an elevated preoperative CEA level is associated with poor prognosis in patients curablly operated upon. It has been reported that CEA positivity ranged from 68.1% to 97.3% at the time when clinical diagnosis of recurrence was made after surgery.[7] Postoperative sequential CEA measurements are a useful

adjunct in clinical follow-up study after surgery. and provide an earlier detection of recurrent cancer.

In view of the mode of recurrence, CEA rose precedingly and simultaneously metastases in the liver, whereas in local recurrence it elevated slowly or remained normal. These findings suggest that serum CEA level may be influenced by production of CEA from the tumor cells and vascularity surrounding recurrent lesions. However, there is no knowledge on the mode of CEA transfer from the tumor mass to the serum. The following factors may be responsible for the alternation of the CEA level : a) total mass present b) ability of tumor cell to produce CEA, c) release rate of synthesized CEA from fumor cells, d) degradation rate of CEA,[9] and e) presence or absence of necrosis in tumor tissues.[8]

Quantitative studies of extracts of primary and metastatic cancers of colon and rectum have shown a wide variation in production of CEA. There is general agreement about CEA in tissue that the CEA concentration is higher in cancerous tissue including primary and metastatic cancer, but very low in non-cancerous tissue.[4][5][10][11] Our data support these findings that cancerous tissues of primary tumors and metastatic tumors into the liver or the lymphnode contain very higher amounts of CEA rather than various non -cancerous tissues.

As for tumor cell differenciation, the CEA content in well and moderately differentiated adenocarcinomas is higher than that in poorly ones. When CEA levels are compared between tumor extract and corresponding serum CEA, there are not statistically significant differences. Of interest is the fact that tumor-producing ability is not associated with elevation of serum CEA in patients with colorectal cancers.

On the other hand, it was noted in the present study that there was a close correlation between the serum level of CEA and the pathological factors of the tumor such as depth of cancer invasion, size of tumor mass, mode of INF and histologic vascular invasion. It has been considered that CEA produced by cancer cells mostly drains into the colonic lumen.[1][8] However, if cancer grows and involves the vessels, large amounts of CEA may be drived into the blood stream. The present study indicate that the pathological findings are a major contributing factor to CEA transfer into blood circulation rather than production of CEA by the tumor.

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