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□ Original Article □

CEA Expressions in Colorectal Tumor

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Purpose: The purpose of this research is to investigate the clinical usefulness of carcinoembryonic antigen (CEA) expression in colorectal cancer tissue.
Methods: We performed immunohistochemical staining of CEA on 64 surgically resected colorectal cancer tissues obtained during the period from May 2000 to May 2001. CEA expression was detected by immunohistochemistry using a CEA monoclonal antibody. The degrees of CEA expression in the tumor cell cytoplasm and the luminal secretion of the tumor gland were grouped into positive (strongly positive) and negative groups (weakly positive) by using the Sinicrobe method and were compared with clinicopathological variables.
Results: The expression rates were positive in 38 cases (59.4%) and negative in 26 cases (40.6%). The preoperative CEA level showed a higher trend in the positive group (8.23±13.7) than it did in the negative group (17.89 ±38.7 ng/ml), but the difference was not statistically significant. The relationships between the CEA expressions of the two groups and the clinicopathologic factors were not statistically significant. We observed CEA expression in the luminal secretion of the tumor gland in 41 cases. The expression rates in the luminal secretion were positive in 21 cases (51.2%) and negative in 20 cases (48.8%). No significant clinical difference were noted between the two groups.
Conclusions: The results suggest that CEA expression may not play a role as a prognostic factor for colorectal cancer.
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Key Words: Carcinoembryonic antigen (CEA), Colorectal neoplasms
암태아성항원(CEA), 대장암

INTRODUCTION

Carcinoembryonic antigen (CEA) is one of the oncofetal antigens that are normally present during fetal life and that are found at low levels in adults. CEA examination has been one of the most important tools for treating colorectal cancer patients, and serial postoperative serum carcinoembryonic antigen has been extensively studied for detecting recurrence and metastasis in patients with colorectal cancer. A continuous increase in the CEA level after colorectal cancer surgery is highly related to tumor recurrence or distant metastasis.[1,2] However, many histopathological parameters, including the serum CEA, have been studied to evaluate their clinical values in the management of colorectal cancer patients, but no satisfying results have been obtained. The weak point of CEA examination is that CEA is not strictly specific for the epithelial cells of colorectal cancer and sometimes leads to false positive or negative results.[3,4] Accurate postoperative prognostic parameters are needed to determine which patients have more risks for recurrence or metastasis after colorectal cancer surgery. Although several studies have suggested a relationship between the CEA immunohistochemical stain and colorectal tumor activity, the effect of the result is still unclear. Thus, we would like to determine whether the presence of CEA immunoreactivity in tumor tissues can be a useful guide for postoperative recurrence of colorectal cancer. The present study was designed to confirm the clinical significance of CEA immunoreactivity in colorectal cancer tissues.

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MATERIALS AND METHODS

We took blood samples from the peripheral veins of 64 patients who underwent operations for colorectal cancer from May 2000 and May 2001 at Dongsan Medical Center, Keimyung University. Cancer samples containing normal mucosa were obtained at the time of surgical resection and were put into formalin immediately and kept at room temperature.

Preoperative serum CEA levels were determined for all patients prior to surgery. CEA levels were measured by doing an enzyme-linked immunoassay with a commercially available kit. We investigated the relationship between the expression of CEA and clinicopathological features in colorectal cancers. The degrees of CEA expression in tumor cells and in gland luminal secretions were divided into positive and negative groups by using the Sinicrobe method (Fig. 1, 2). The time to recurrence was defined as the disease free interval until the recurrence.

1) Immunohistochemistry

Sialanized slides (DAKO code No. 3003) were used for adhering tissue sections to glass slides, and the tissue sections were placed in a water bath at 59°C for 30 min prior to the immunohistochemical staining procedure.

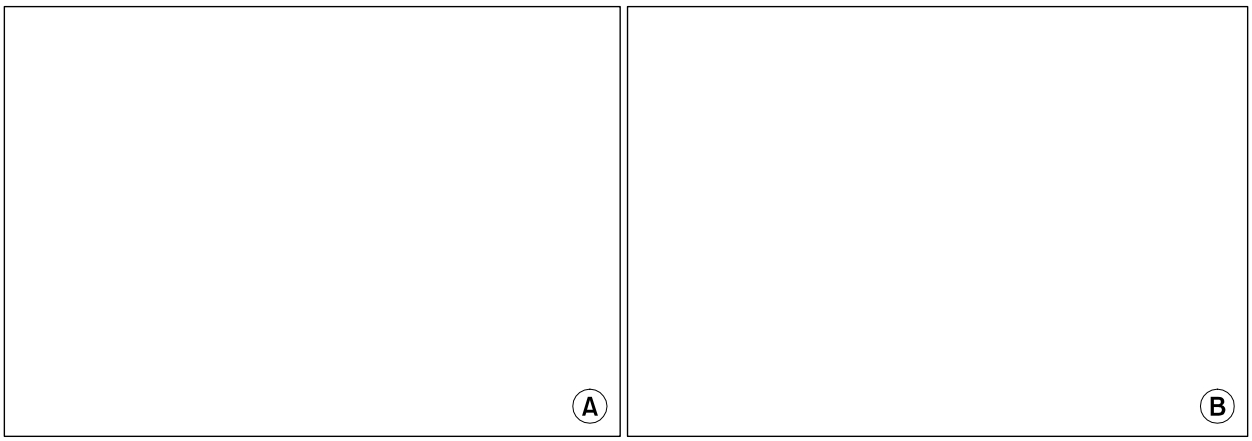

Fig. 1. CEA immunohistochemical staining patterns for colorectal cancer tissue: (A) positive pattern with high CEA immunoreactivity (brown color) is demonstrated in the cytoplasm (×100). (B) negative pattern with Low CEA immunoreactivity (×100).

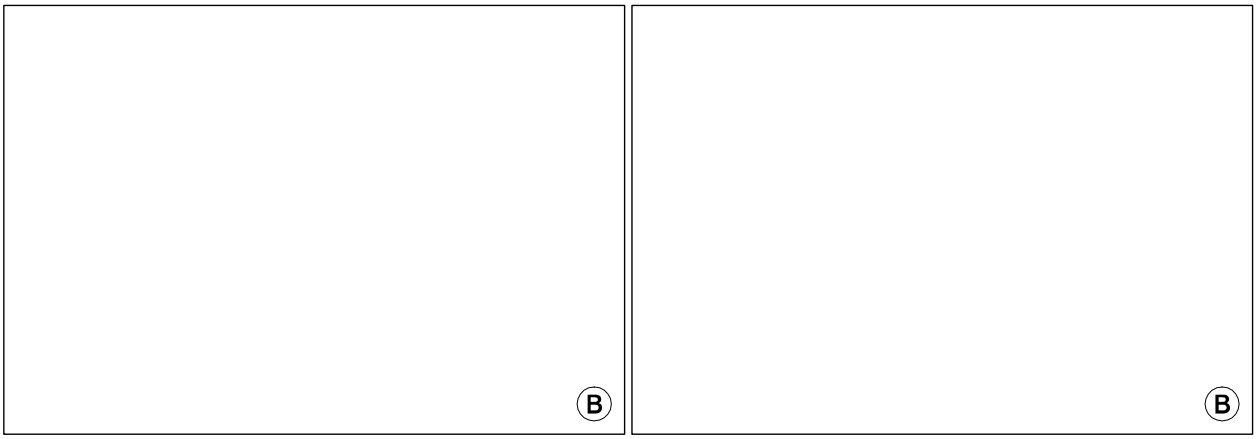

Fig. 2. CEA immunohistochemical staining pattern for secretive fluid in the lumen of colorectal cancer tissue: (A) positive pattern with high CEA immunoreactivity (brown color) demonstrated in the lumen (×100), and (B) negative pattern with low CEA immunoreactivity (×100).

Before using the water bath, we preheated a coplin jar containing the preheated buffer and heated the tissue sections for 40 min. After the paraffin had been removed by heating, the tissue sections were exposed to xylene 3 times in 5 min. The sections were treated with 100%, 95% (3 times), 80%, and 70% ethanol for 5 min, respectively. The sections were pre-incubated for 20 min with 3% hydrogen peroxide to block endogenous peroxidase activity; then, the slides were rinsed 3 times in phosphate buffered saline (PBS). The slides were then auto-claved in a citrate buffer (PH 6.0) for 10 min and cooled to room temperature for 20 min. After the slides had been washed 3 times in 5 min, the sections were incubated with primary antibody CEA for an hour in a humid chamber at 37°C and rinsed with PBS 3 times. The antibody was no longer used in the diluted state. The samples were re-incubated with a secondary antibody (DAKO LSAB, Monoclonal mouse anti-human carcinoembryonic antigen, 11-7) for 20 min at the room temperature. After the sections had been washed with PBS 3 times in 5 min, they were incubated for 20 min with streptavidin peroxidase. They were then counter-stained with hematoxyline. After neutralization, they were treated with ethanol and xylene. Negative controls were stained without the primary antibody by using the same method as above.

2) Grading of Immuno-stained Slides

Each slide was viewed, not knowing the source of the tissue and the outcome of the patient. The tumor on the entire slide was inspected. The grades of staining for CEA revealed a score of 1 to 4 for both intensity and distribution (Table 1). The final score was calculated in terms of the intensity multiplied by the expression rate. A final score above 4 was expected to be positive and one below 4 to be negative.

Table 1. Quantification of immunostaining for CEA (Sinicrobe method)

Score	Intensity of staining	Expression rate
1	Weak	＜25%
2	Moderate	25～50%
3	Strong	50～75%
4		75～100%

3) Follow-up of Patients

The patients were managed according to the follow-up schedule of our department. All patients except 4 (60/64) were subjected to a 2-year follow-up after surgery. The follow-ups were done every 2 months during the first year and every 3 months during the second year. Two years after the operation, the status of every patients regarding recurrence were evaluated. Every evaluation consisted of the patients' history, the serum CEA, liver function test including liver ultrasonography in every 2 or 3 months, annual colonoscopy, annual abdominal computed tomography and annual chest PA.

4) Statistical Analysis

The statistical analyses were performed with the SPSS of the windows program package. The statistical analysis was defined using the Chi-square test and the Student's t test. The advancement of the cancer stage was recorded according to the TNM staging system for colorectal cancer. The disease free time was recorded as the number of months from the day of surgery. Statistical significance was defined as a P value of less than 0.05.

RESULTS

Most of the staining represented by brown coloring was

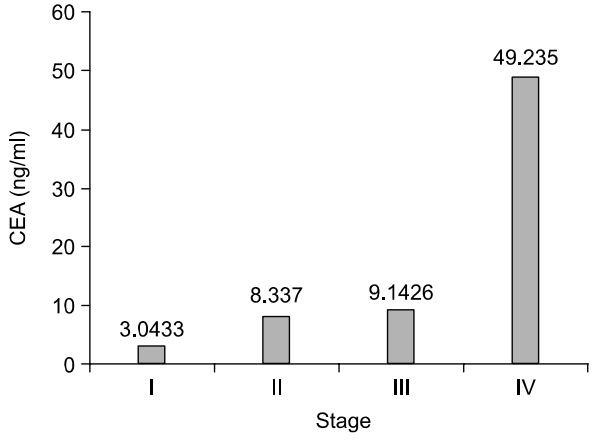

Fig. 3. Relationship between the mean serum CEA level and the stage (P=0.024).

observed partially on the cytoplasm, and secretive fluid was found in the lumen of the tumor gland. Although a slight weak staining was observed on the entire normal colonic mucosa, staining was not observed in the stromal cells. The sites of the staining were similar in each primary lesion, but their intensities and staining rates were different. The expression rates were positive in 26 cases (40.6%) and negative in 38 cases (59.4%). The mean levels of preoperative serum CEA were 3.04 ng/ml, 8.34 ng/ml, 9.14 ng/ml and 49.24 ng/ml in stages 1, 2, 3, and 4, respectively, and it was correlated according to the TNM stage, with a statistical significance of P=0.024 (Fig. 3).

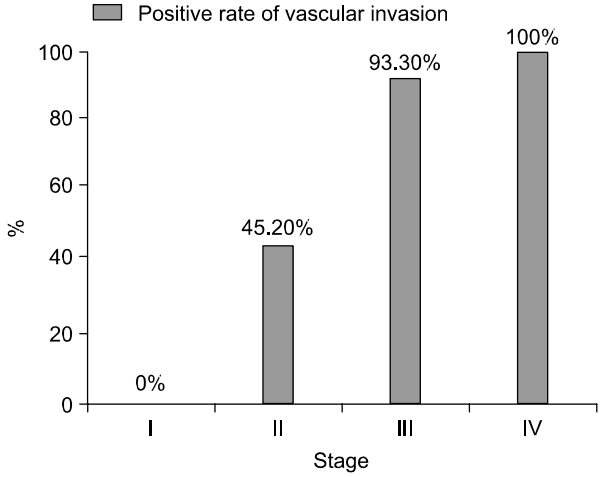

Fig. 4. Relationship between the positive rate of vascular invasion and the stage (P=0.02).

Data on vascular invasion from pathologic reports were available 51 cases, and vascular invasion was more frequently observed in the advanced stage of the tumor (P=0.002)(Fig. 4). The preoperative CEA level showed a higher trend in the positive group than it did in the negative group (17.89±38.7 vs. 8.23±13.7 ng/ml), but the difference was not statistically significant (Table 2). No correlation was found between the CEA staining rate and other clinicopathological parameters, including the recurrence rates (Fig. 5～7).

DISCUSSION

Despite the remarkable advances in the treatment of colorectal cancer, the serious problems of recurrence and metastasis require further research to be resolved. The classic tumor marker CEA has been considered to be one of the most useful tools for monitoring colorectal cancer recurrence. CEA is a glycoprotein that is present on the

Table 2. Relationship between CEA immunohistochemical staining and the mean serum CEA level

CEA immunohisto-chemical staining	Mean serum CEA level (ng/ml)
Positive (26 cases)	17.89±38.7
Negative (38 cases)	8.23±13.7

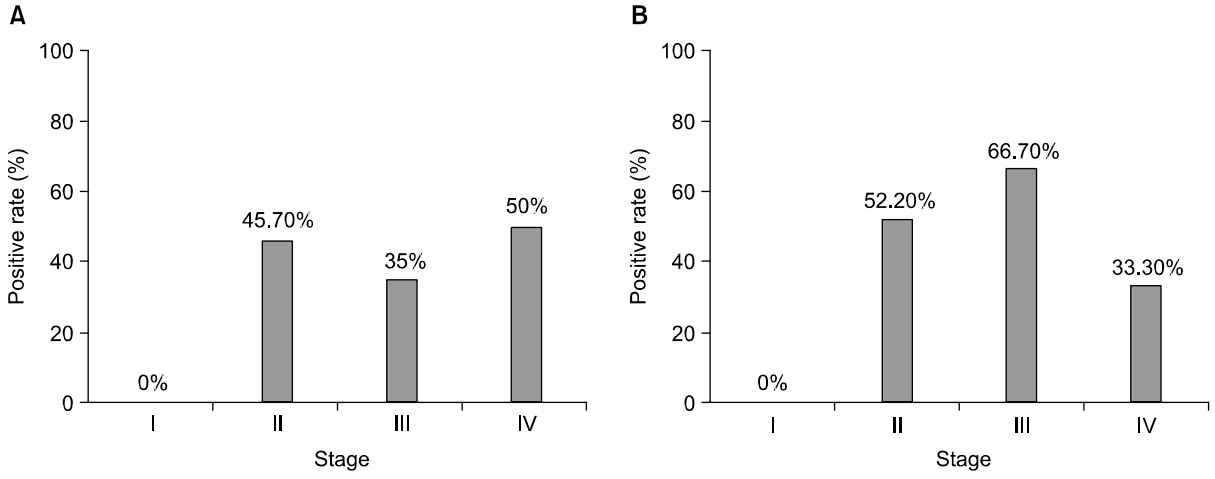

Fig. 5. Relationship between the positive rate of CEA immunohistochemical staining and the stage: (A) cytoplasmic CEA staining (P＞0.05), and (B) luminal CEA staining (P＞0.05)).

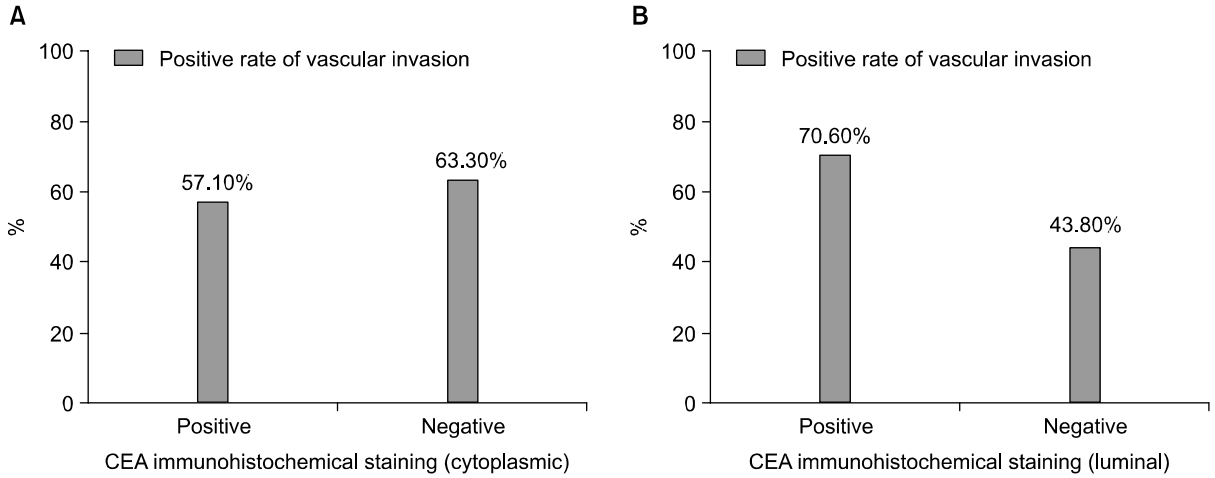

Fig. 6. Relationship between the results of CEA immunohistochemical staining and vascular invasion: (A) cytoplasmic CEA staining ($P > 0.05$), and (B) luminal CEA staining ($P > 0.05$)).

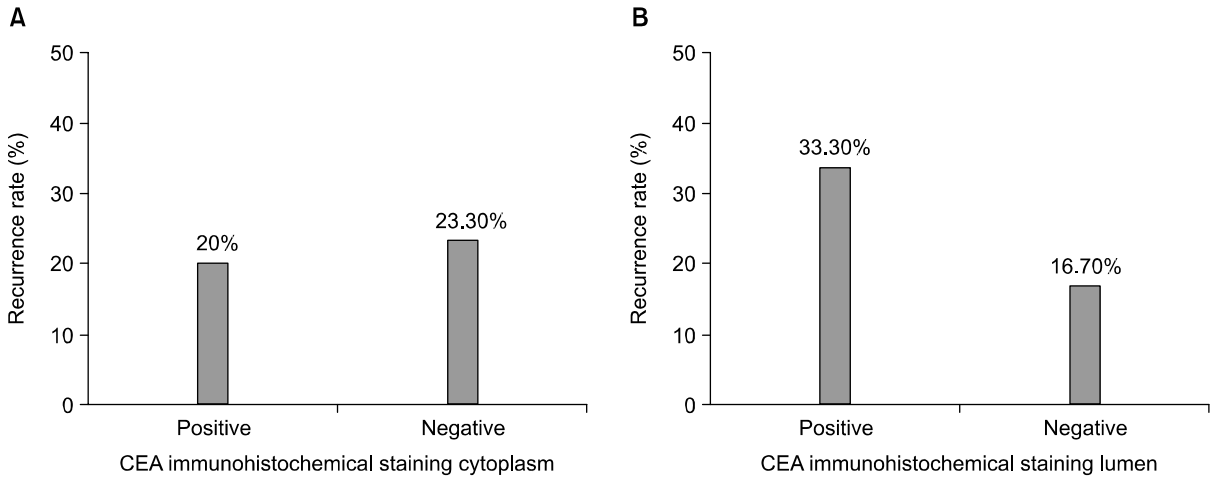

Fig. 7. Relationship between the CEA immunohistochemical staining and the recurrence rate: (A) cytoplasmic CEA staining ($P > 0.05$), and (B) luminal CEA staining ($P > 0.05$)).

cell surface.[5,6] Since CEA was first described in 1965,[5,6] it has aroused much interest among colorectal surgeons and has been one of the most widely used tumor-marker antigens. Because most colorectal cancer recurrence occurs within 2 years after the operation, tumor recurrence and metastasis should be detected early in order to improve the results of reoperation and the survival rate.

An elevated preoperative CEA level has been considered as an indicator for high risk of recurrence and poor prognosis after colorectal cancer surgery.[7-9] The postoperative CEA value has given information on cancer recurrence, but rarely has brought about better outcomes in colorectal cancer patients.[3]

CEA is not strictly specific to epithelial cells of colorectal cancer, so it sometimes leads to false positive results.[10-12] CEA released from cancer cells enters the liver through portal blood. It is diluted and degraded in the liver and gives a CEA value at peripheral vessels. Thus, we cannot get exact information about a patient's status by just measuring the postoperative CEA level of the peripheral blood.

Despite the problem of the non-specificity of the CEA assay, it is still an object of study. In addition, at the moment, there is no other marker to substitute for CEA

in detecting colorectal cancer recurrence. Since we don't have the best and the easiest methods to detect recurrence, we need to have parameters to predict the recurrence of a tumor and non-invasive, easy tools to examine the recurrence after the operation. Therefore, we need to know whether the presence of CEA immunoreactivity in tumor tissues will trigger clinical aggressiveness in a patient's individual tumor. Our work showed that all the primary colorectal cancer lesions and the normal colonic mucosa were positive to this monoclonal antibody, but the intensities and the sites of the staining were slightly different. For the same patients, the expression of CEA was slightly higher in cancer tissues than it was in the normal colonic mucosa. CEA staining was mainly found within the cell cytoplasm; the secretive fluid in the gland lumen of the tumor tissue was partially stained.

Hamada et al.[13] reported that vascular and lymphatic invasions were observed much more frequently in cases of cytoplasmic- and stromal-type tumors than they were in apical-type tumors. Teixeira et al.[14] found that more cases of cytoplasmic patterns penetrated through the bowel wall, but we found no relation between the sites of staining with a CEA antibody and the level of penetration.

The prognostic significance of the intensity of tumor CEA expression has not been established. We also found no relationship between the rate of CEA expression and the activity of the colorectal tumor cell. We found that the extent of CEA expression in colorectal cancer tissue did not affect the preoperative serum CEA level or the disease-free survival after the operation.

There has been much controversy over whether stromal CEA adjacent to carcinoma cells might be an important factor in the elevation of serum CEA levels in colorectal cancer.[13,15,16] Nagura et al.[17] speculated that CEA on basolateral surfaces of malignant cells, where it might have easy access to the surrounding stroma and the blood circulation, might account for the elevated levels of CEA in the serum of gastric cancer patients. However, we did not find the expression of stromal CEA or any casual relationship between the clinicopathologic variables and the amount of tumor CEA expression. For applications of different CEA antibody and experimental techniques, the results have turned out to be different.

It is not clear at the moment if the reason for the negative result is a defect in the method and if it would be possible to get a positive result by using other antibodies or techniques. CEA is not the major cause of tumor recurrence. Some of the proteins associated with invasion and metastasis may be secreted by the tumor cells. There are also many factors associated with the complex cascade of the tumor recurrence that are still unknown.

In summary, though we have not compared survival rates between the high- and low-expression groups, the results presented in this study suggest that CEA staining on cancer tissue due to immunohistochemical staining may not play any role as a clinical prognostic marker for colorectal cancer patients. Further studies involving a larger number of cases and using various antibodies for CEA and other cell-surface glycoproteins are necessary to evaluate theefficacy of CEA staining in obtaining a prognosis for colorectal cancer patients.

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국문 초록

대장암에서 CEA의 발현

계명대학교 의과대학 외과학교실 대장항문 분과
배옥석 · 이태순 · 박성대

목적: 종양조직내의 CEA 발현의 정도가 환자의 예후 예측을 위한 탐지자로서의 가능성 유무를 확인하고자 본 연구를 시작하였다.
대상 및 방법: 2000년 5월부터 2001년 5월까지 계명대학교 동산의료원 대장항문과에서 결직장암으로 수술한 환자에서 획득한 암조직 64예를 CEA항체를 이용하여 면역조직화학염색을 시행하여 암세포와, 관강 내 분비물의 발현양상을 양성 음성으로 구분후 각 환자들의 술 전 혈청 CEA치, 병기와 혈관 침범유무, 술 후 2년 내 암재발과의 관계를 분석하였다.
결과: 64예의 암조직의 발현은 양성 26예, 음성 38예였으며 술전 말초혈액 CEA치와 비교에서 음성과 양성은 각각 8.23±13.7, 17.89±38.7 ng/ml로 양성군에 증가하였으나 통계적 유의성은 없었으며 병기, 혈관침범유무, 재발 등과의 비교에서도 유의한 차이는 없었다. 관강 내 분비물의 발현에서도 임상적인 조건들과의 유의한 관계가 없었다.
결론: 대장암에서 CEA의 발현은 예후인자와의 상관관계를 확인할 수 없어서 환자의 예후예측을 위한 탐지자로서의 임상적 의의가 없는 것으로 사료된다.