

Significance of correlation between levels of carcinoembryonic antigen and carbohydrate antigen 19-9, carcinoembryonic antigen and C-reactive protein, carcinoembryonic antigen and alpha-1 antitrypsin in gastric and colon cancer patients

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ABSTRACT

Aim: Recent progress in proteomics studies profiled that serum proteins of cancer patients and those of normal individuals have altered cancer antigen and acute phase protein expression for distinct types and stages of cancer. In our study, correlation between carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9, CEA and C-reactive protein (CRP), CEA and alpha-1 antitrypsin (A1AT) were evaluated in gastric and colon cancer patients. **Materials and Methods:** CEA was estimated by solid phase, two-site sequential chemiluminescent immunometric assay, CA19-9 by solid phase enzyme-linked immunosorbent assay, CRP by latex turbidimetry method and A1AT by turbidimetry method. **Results:** A significant correlation was seen in levels of CEA and CA19-9 in gastric ($r = 0.457, P < 0.001$) and colon cancer ($r = 0.451, P < 0.001$) patients. Correlation between CEA and CRP was significant in gastric ($r = 0.462, P < 0.001$) and colon cancer ($r = 0.759, P < 0.001$) patients and between CEA and A1AT also, correlation was found to be significant in gastric ($r = 0.631, P < 0.001$) and colon cancer patients ($r = 0.516, P \leq 0.001$). **Conclusion:** Serum acute-phase protein concentrations, when combined with CEA increases the sensitivity of CEA and provide substantial information concerning the diagnosis of gastrointestinal cancers. They have a definite role as a significant prognostic indicator which undoubtedly correlates with progression of cancer. Combined CEA and CA19-9 positivity reflected more biologic malignant properties and were significantly correlated with lymph node metastasis, hepatic metastasis and lower rates of curative resection. Surgical outcomes of patients who were CEA and CA19-9 positive were poorer than those of patients with normal CEA and CA19-9 levels.

Key words: Alpha-1 antitrypsin, carbohydrate antigen 19-9, carcinoembryonic antigen, colon cancer, C-reactive protein, gastric cancer

INTRODUCTION

The terms cancer, neoplasm, tumor and malignancy are usually used interchangeably. Malignancy has been defined

by Sir Rupert Willis "as a group of abnormal mass of tissues, the growth of which far exceeds, un-coordinated with that of the normal tissues and persists in the same excessive manner after cessation of stimuli which evoked the change."^[1] Tumor markers are molecules occurring in blood or tissue that are associated with cancer and whose measurement or identification is useful in patient diagnosis or clinical management. These markers may be detected within exfoliated or distributed cells, or as circulating agents within the peripheral blood or plasma. Other surrogate biological specimens, typically bodily fluids (e.g., urine, saliva, sputum, cerebrospinal fluid, or effusions) may also

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carry tumor markers.^[2] Acute-phase proteins are a class of proteins whose plasma concentrations increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction (also called acute-phase response). The liver responds by producing a large number of acute-phase reactants: “positive” acute-phase proteins serve (part of the innate immune system) different physiological functions for the immune system. Some act to destroy or inhibit the growth of microbes, e.g., C-reactive protein (CRP), mannose-binding protein, complement factors, ferritin, ceruloplasmin, serum amyloid A and haptoglobin. Alpha-1 antitrypsin (A1AT) and alpha 1-antichymotrypsin are positive acute-phase proteins (serpin), which down regulates inflammation. “negative” acute-phase proteins decrease in inflammation e.g., albumin, transferrin, transthyretin, etc., The decrease of such proteins may be used as markers of inflammation. The physiological role of decreased synthesis of such proteins is to save amino acids for producing “positive” acute-phase proteins more efficiently.^[3]

Aims/objectives

In view of the compelling association of different patterns of tumor markers and acute phase reactant proteins with various types of cancers, the study was planned to evaluate their prognostic significance in gastrointestinal cancer patients.

Serum levels of tumor markers and acute phase reactant proteins in cancer patients before therapy were compared with normal healthy subjects. Correlation between carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9, CEA and CRP, CEA and A1AT were evaluated in gastric and colon cancer patients.

MATERIALS AND METHODS

Clinical data

The subjects included 100 patients suffering from gastric and colon cancer diagnosed by endoscopic examination and biopsy and who have not received any anticancer therapy before. Fifty healthy subjects with no cancer comprised the normal control group. The hematological and biochemical profile of each cancer patient and each healthy subject was evaluated. All patients and healthy control subjects were recruited from the Department of Radiotherapy, SMS Medical College and Hospital, Jaipur from July 2011 to December 2012. All subjects were screened with a specially designed screening proforma which encompassed both inclusion and exclusion criteria. The study was approved by the Institutional Research Committee. Written informed consent form was obtained from all patients and healthy subjects.

Inclusion criteria

- Healthy subjects were identified as individuals not suffering from any physical ailment or acute illness, not hospitalized for any disease in the past 2 years and not addicted to smoking, tobacco, or alcohol Consumption
- Patients were identified as individuals suffering from gastric and colon cancers currently diagnosed by endoscopic examination and biopsy and have not received any anticancer therapy before.

Exclusion criteria

- Healthy subjects with any type of gastrointestinal infections such as gastroenteritis, diarrhea, dysentery, enterocolitis, suffering from acute illness, hospitalized recently, or addicted to smoking, alcohol, or tobacco consumption were excluded from this study.
- Cancer patients who have received radiotherapy or chemotherapy (those who started the treatment recently or were old patients receiving treatment from several months back) or undergone any surgery were excluded.

Study design

Clinical history

Each patient was first examined by obtaining a brief clinical history related to diet, life-style, initial symptoms, or any previously received treatment.

The patients and healthy subjects were categorized as:

- Group 1:50 normal healthy subjects
- Group 2:50 patients with gastric cancer
- Group 3:50 patients with colon cancer.

Sample collection and analysis

Blood samples were collected prior to administering any therapy in gastrointestinal cancer patients and as a part of routine investigation in healthy subjects. The samples were collected in plain vial and allowed to clot. Serum was separated by centrifugation at 3000 rpm or 3485 ‘g’ for 10 min and stored at -20°C until further assay was performed.

- CEA was estimated by solid phase, two-site sequential chemiluminescent immunometric assay,
- CA19-9 by solid phase enzyme-linked immunosorbent assay,
- CRP by latex turbidimetry method and
- A1AT by turbidimetry method.

The tests were performed strictly according to the manufacturer’s instructions and as stated in the literature. Frequent false-positive outcomes result from benign gastrointestinal disorders and smoking. Thus, the threshold values for CEA in GI cancers according to the kit were as follows: Male smokers: 6.2 ng/mL; male non-smokers: 3.4 ng/mL; female smokers: 4.9 ng/mL; female non-smokers:

2.5 ng/mL; CA19-9 assay values below 35 U/mL (for healthy men and women); CRP values up to 5 mg/L for healthy men and women; A1AT values 90-200 mg/dL for healthy men and women.

RESULTS

Data were analyzed using SPSS version 10.0 (SPSS Inc., USA) and MedCalc to estimate the significance of the observed differences and find out the correlation. Correlation levels were analyzed by Pearson correlation coefficient (*r*).

A significant correlation was seen in levels of CEA and CA19-9 in gastric ($r = 0.4571, P < 0.001$) and colon cancer ($r = 0.4519, P < 0.001$) patients. Correlation between CEA and CRP was significant in gastric ($r = 0.4625, P < 0.001$) and colon cancer ($r = 0.7593, P < 0.001$) patients. Correlation between CEA and A1AT was also significant in gastric ($r = 0.6315, P < 0.001$) and colon cancer patients ($r = 0.5168, P \leq 0.001$) [Table 1, Figures 1-6].

DISCUSSION

Correlation between CEA and CA19-9 in gastrointestinal cancer patients

In our study, correlation between CEA and CA19-9 were evaluated in gastric and colon cancer patients. Our results were similar to Ueda *et al.*, who observed that serum CA19-9 levels were well-correlated with serum CEA levels in colon cancer patients.^[4] In gastric cancer patients similar findings were seen in the study of Ishigami *et al.*, who observed that serum CEA and CA19-9 positivity significantly correlated with depth of invasion, hepatic metastasis and curativity. Patients positive for both CEA and CA19-9 had significantly higher frequencies of lymph node metastasis, deeper invasion by the tumor, lower rates of curative resection ($P < 0.01$) and higher rates of hepatic metastasis ($P < 0.05$) compared with patients with normal levels of CEA and CA19-9. Surgical outcomes of patients who were CEA and CA19-9 positive were poorer than those of patients with normal CEA and CA19-9 levels ($P < 0.01$). In view of the combined CEA and CA19-9 positivity, the tumors reflected more biologic malignant properties.^[5]

Correlation between CEA and CRP in gastrointestinal cancer patients

In our study, correlation between CEA and CRP were evaluated in gastric and colon cancer patients. A study by Nozoe *et al.*, investigated the correlation between elevated pre-operative serum CRP level and clinicopathologic factors including prognosis of 116 patients who underwent resection of CRC. They reported close correlation between elevated pre- or post-operative serum CRP and tumor progression in patients with colorectal carcinoma. Moreover, the importance of serum elevation of CRP in the tumorigenesis and pathogenesis of CRC has become a focus of much attention among patients whose serum values of both CRP and CEA had been measured pre-operatively. A high serum CRP level was significantly correlated with a high serum CEA level. There is an established significant correlation between the stage of tumors and elevated serum CRP ($P < 0.0001$) and CEA ($P = 0.006$) but an elevated serum CRP level demonstrated more powerful stratification. The existence or elevation of serum CRP in patients with malignant tumors might be clinically important for two reasons: Firstly, it is a common theory that CRP is increasingly synthesized by the hepatocytes as a host response to the malignant process. This suggests that the malignant tumor is in fact an inflammatory disease. Second, the tumor cells might produce CRP, like macrophages and lymphocytes, which are involved with host defense. This study was motivated by the established result that cytokines such as interleukin-6 and tumor necrosis factor up regulating the synthesis of CRP are expressed in tumor cells and play a role in tumor progression. The elevation of serum CRP is undoubtedly correlated with the progression of CRC and elevated serum CRP level was significantly correlated with an elevated serum CEA level. Some investigators have reported that CRP could promote the host immune defense including the proliferation of lymphocytes. Conversely, previous study demonstrated that the elevation of serum CRP was significantly correlated with a decreased lymphocyte count in the peripheral blood of patients with CRC, suggesting the possible correlation of an elevated serum CRP level with impaired immunity in tumor-bearing patients. These investigations indicated that the function of CRP may be diverse in the immune system of tumor-bearing hosts.^[6]

Table 1: Correlation coefficient (*r*) between CEA and CA19-9, CEA and CRP, CEA and A1AT in gastric and colon cancer patients

	Gastric cancer			Colon cancer		
	Pearson correlation coefficient (<i>r</i>)	<i>P</i>	Significant/NS	Pearson correlation coefficient (<i>r</i>)	<i>P</i>	Significant/NS
CEA and CA19-9	0.457	0.000	Extremely significant	0.451	0.000	Extremely significant
CEA and CRP	0.462	0.000	Extremely significant	0.759	0.000	Extremely significant
CEA and A1AT	0.631	0.000	Extremely significant	0.516	0.000	Extremely significant

* $P < 0.05$ is significant. CEA: Carcinoembryonic antigen, CRP: C-reactive protein, A1AT: Alpha-1 antitrypsin, CA19-9: Carbohydrate antigen 19-9

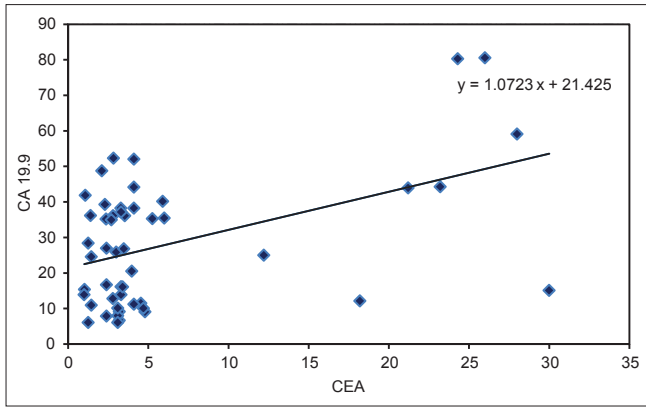


Figure 1: Correlation of carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9) in gastric cancer. *Correlation between CEA and CA19-9 was significant in gastric cancer patients ($r = 0.4571, P < 0.001$)

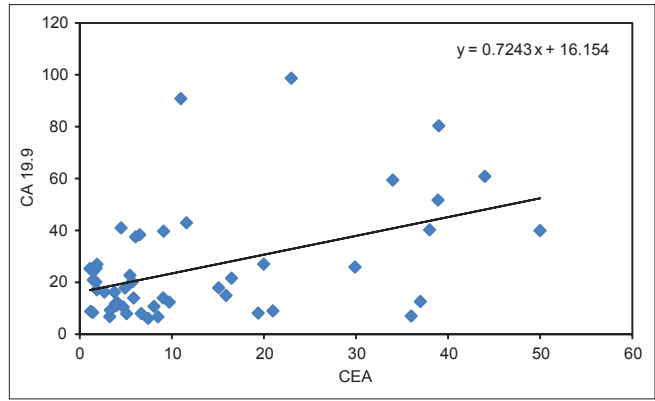


Figure 2: Correlation of carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9) in colon cancer. *Correlation between CEA and CA19-9 was significant in colon cancer patients ($r = 0.4519, P < 0.001$)

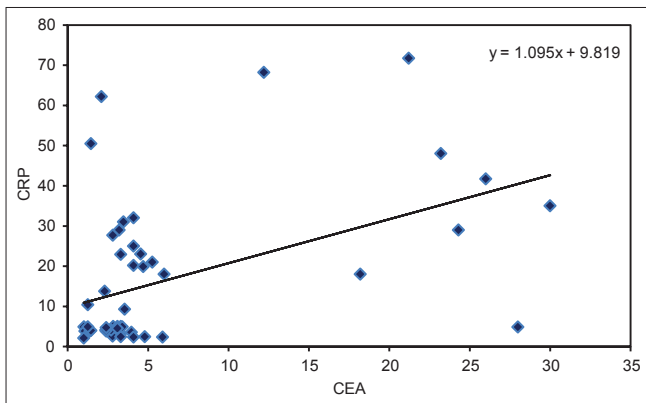


Figure 3: Correlation of carcinoembryonic antigen and C-reactive protein in gastric cancer. *Correlation between CEA and CRP was significant in gastric cancer patients ($r = 0.4625, P < 0.001$)

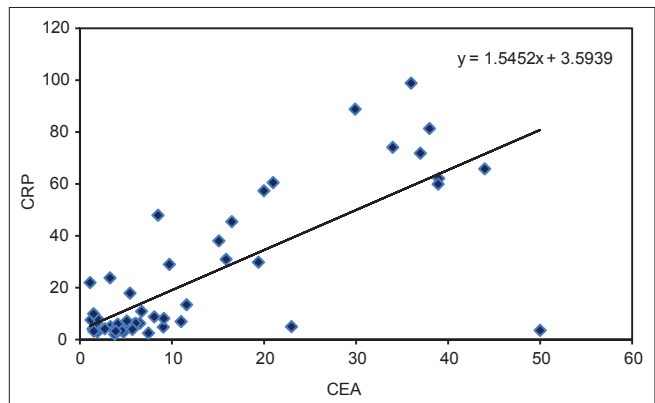


Figure 4: Correlation of carcinoembryonic antigen and C-reactive protein in colon cancer. *Correlation between CEA and CRP was significant in colon cancer patients ($r = 0.7593, P < 0.001$)

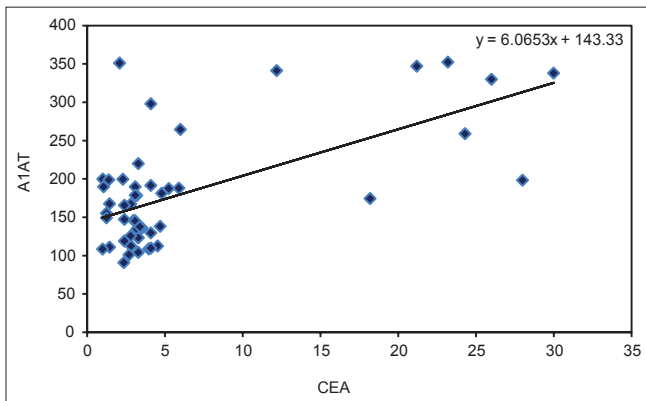


Figure 5: Correlation of carcinoembryonic antigen and alpha-1 antitrypsin (A1AT) in gastric cancer. *Correlation between CEA and A1AT was significant in gastric cancer patients ($r = 0.6315, P < 0.001$)

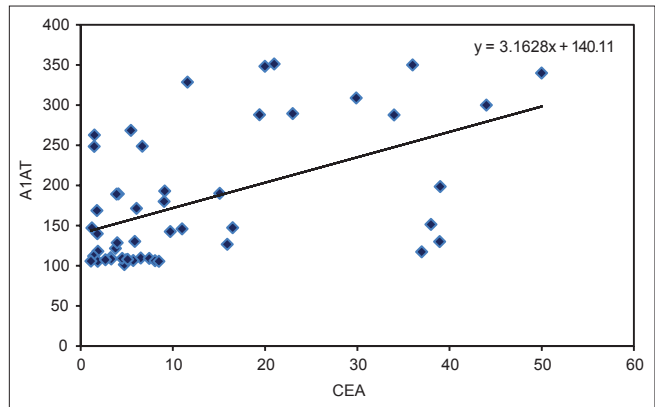


Figure 6: Correlation of carcinoembryonic antigen and alpha-1 antitrypsin (A1AT) in colon cancer. *Correlation between CEA and A1AT was significant in colon cancer patients ($r = 0.5168, P \leq 0.001$)

Wigmore *et al.*, evaluated CRP concentration, before and at 3 months after operation, which was used as an index of the APRP. Univariate and multivariate analysis were performed on a number of potential prognostic factors. Nearly 36% of patients had high APRP associated with a higher rate of local tumor invasion, fewer curative resections and a higher CEA concentration.^[7]

Correlation between CEA and A1AT in gastrointestinal cancer patients

In our study correlation between CEA and A1AT were evaluated in gastric and colon cancer patients. Solakidi *et al.*, observed elevated levels of tumor-associated trypsin inhibitor (TATI) in 50% and 41.7% of patients with gastric and colorectal cancer, elevated levels of TATI were

observed only in 8% of patients with benign gastrointestinal malignancies (92% specificity). Elevated levels of CEA were observed in 25% and 24.4% of patients, respectively. The total positivity of CEA and TATI (with at least one marker positive) was 62.5% and 57%, respectively. Spearman's test has shown a statistically significant correlation among serum TATI, CRP and A1AT levels ($P < 0.01$).^[8]

A1AT is a serum glycoprotein mainly synthesized in human liver cells and macrophages. It is one of the member of serpins family, which plays a central role in controlling tissue degradation through its inhibitory effect on neutrophil elastase and other serine proteases including: trypsin, chymotrypsin, cathepsin G, plasmin, thrombin, tissue kallikrein and activated factor X. These proteins constitute the third major protein component of blood plasma after albumin and immunoglobulins.^[9] It was demonstrated that many types of tumor cells are capable of expression and secretion of A1AT and the major source of the increased A1AT blood levels in cancer patients is the growing cancer cells.^[10]

Stamatiadis *et al.*, found in 55 patients with benign or malignant neoplasia of large bowel, serum CEA, CRP, A1AT, Alpha 1-Acid Glycoprotein (AAG) levels and the percentage of serum protein electrophoretic components were measured. Statistical analysis showed significant correlations between serum CEA, CRP, AAG and A1AT levels and the percentage of serum beta-globulins with the stage of disease. The authors concluded that the serum acute-phase protein levels in combination with serum CEA concentrations have a definite role in the pre-operative staging of large bowel cancer. Many authors reported that serum acute-phase protein concentrations especially when combined with serum CEA provided substantial information concerning the diagnosis of gastrointestinal tract cancer. The combination of CEA with isolated acute-phase reactants or a profile of such parameters increased the sensitivity of CEA in the diagnosis of colorectal cancer.^[11] Bernacka *et al.*, in their study have reported a similar but less significant correlation coefficient (r) concerning A1AT levels ($r = 0.353$, $P < 0.01$). The actual role of the high levels of acute-phase reactants in cancer-bearing patients is that these antiproteases might inhibit tumor-produced enzymes that help in the invasion of surrounding tissues. In patients with a locally limited neoplasia and vigorous peritumoral inflammatory reaction, very high serum concentrations, probably denoting a relatively good prognosis, were found.^[12] Contradictory report was given by Yuceyar *et al.*, they suggested the role of acute-phase reactant proteins in combination with CEA and CA19-9 at the pre-operative staging of colorectal cancer. In 22 patients with cancers of the colon and rectum and in 9 control patients without cancer the serum levels of CEA, CA19-9, CRP, A1AT and AAG

were measured. While, statistical analysis did not showed significant correlations between serum CEA, A1AT and CRP levels with the stage of disease, the significant correlations between serum CA19-9 and AAG concentrations with the extent of cancer were detected.^[13]

CONCLUSION

Our study supported the following conclusion:

- Combined CEA and CA19-9 positivity reflected more biologic malignant properties and are significantly correlated with lymph node and hepatic metastasis and lower rates of curative resection. Surgical outcomes of patients who were CEA and CA19-9 positive were poorer than those of patients with normal CEA and CA19-9 levels.
- Serum acute-phase protein (CRP, A1AT) concentration, when combined with CEA increased the sensitivity of CEA suggesting that the malignant tumor is in fact an inflammatory disease and provided substantial information concerning the diagnosis and pre-operative staging of gastrointestinal cancer. They have a definite role as significant prognostic indicator which undoubtedly correlates with progression of cancer.

Using the information that these markers and acute phase proteins can provide, patient-specific treatment protocols can be developed, implemented and monitored for improved patient outcomes.

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