Evaluation of Serum Adenosine Deaminase as a Tumor Marker in Gastric Cancer

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Abstract: Currently recommended tumor markers in gastric cancer are being used to monitor treatment or detect recurrences and serum carcinoembryonic antigen (CEA) is a widely accepted tumor marker in the management of gastric cancer. Hence there is a need for identifying a tumor marker which will be useful for detecting gastric cancer. Alterations in enzyme levels in cancer are well known, and adenosine deaminase (ADA), an enzyme of the purine salvage pathway, has been found to be elevated in various malignancies. The present study was carried out on 30 healthy controls and 22 gastric cancer patients. ADA in serum was estimated by colorimetric method of Galanti and Giusti. The diagnostic accuracy of ADA was assessed by receiver operating characteristics (ROC) curves. The diagnostic relevance of independent and two marker combination was analyzed by logistic regression model. Increase in serum ADA (p < 0.001) and CEA (p < 0.005) activity was found in the patients when compared to controls. Areas under the ROC curve were 0.908 for ADA, 0.865 for CEA. The area under the ROC curve when both ADA and CEA were combined was 0.952. ADA was found to have an independent strong predictor outcome and hence may be considered as a potential tumor marker in gastric cancer.

Key words: Gastric cancer, tumor markers, adenosine deaminase, carcinoembryonic antigen.

INTRODUCTION

Gastric cancer is the second most frequent cancer in the world after lung cancer, with 60% of cases occurring in developing countries and with about 800,000 new cases diagnosed every year [1]. It traditionally carries a poor prognosis with 79% of the tumors diagnosed at an advanced stage, when the five year survival rate is less than 5%. If diagnosed earlier, gastric cancer has 95% cure rate. In contrast, advanced stages are generally refractory to chemotherapy leading to poor prognosis. Lack of a simple, inexpensive, non-invasive and reliable screening test has been quoted as the main reason. Traditional methods of diagnosis include biopsy, barium x-ray, gastroscopy, computer tomography and cytology, all of which are cumbersome and invasive except for barium x-ray. In this scenario minimally invasive cancer specific tests are urgently sought and recently serological tumor markers have been included and actively pursued to obtain an easy, simple, reliable diagnostic tool for the detection of gastric cancer.

Tumor markers are substances that are detected in blood, urine, or body tissues of some patients with certain types of cancer. Most tumor markers can be produced by cancer cells as well as normal cells and may not be elevated in every person with cancer especially in the early stages. Many are not specific to a particular type of cancer as elevated levels are found in more than one type of cancer. For several reasons, tumor markers by themselves are usually not enough to diagnose or rule out cancer. Gastrointestinal tumor markers were developed for screening of colorectal cancer and were also studied in other gastrointestinal malignancies, but no marker has yet been found to be useful for gastric cancer. Carcinoembryonic antigen (CEA) was first used as a specific marker for colonic cancer, but it was also found to be elevated in various other malignancies like stomach, breast, lung, pancreatic, bladder, hepatic cancers, lymphoma and melanoma and also in other benign conditions like cigarette smoking, peptic ulcer disease, inflammatory bowel disease, pancreatitis, cirrhosis and in biliary obstruction [2]. Other tumor markers used in the diagnosis and prognosis of gastric cancer include CA125, CA19-9, CA72-4 and AFP. Among these CEA, CA19-9 and CA72-4 are the three most widely studied tumor markers for their individual or combined predictability, with the main focus being on monitoring treatment to detect recurrence. Serum CEA and CA19-9 are reported to be good prognostic factors in gastric cancer [3]. However none of these markers meet the
original goal of discovering cancer at an early stage and hence the pursuit for newer markers has continued. The National Academy of Clinical Biochemistry (NACB) Guidelines for the Use of Tumor Markers in Gastric Cancer says that the use of markers for the diagnosis of gastric cancer cannot be recommended as none are specific and sensitive enough to be included in the diagnostic procedure [4].

It has been known for many years that the enzyme complement of a tumor cell differs in many ways from that of its normal counterpart reflecting its altered metabolism. Elevated enzyme levels in cancer patients frequently decrease to normal levels following successful treatment, with unchanged or increasing levels indicating a lack of response. Attempts to exploit this information clinically have led to the assay of a wide variety of enzymes in the search for both serum and tissue tumor markers. Serum enzymes are useful for monitoring the effects of therapy, to detect recurrences and also have prognostic value as their level frequently reflects tumor burden. Adenosine deaminase (ADA) an enzyme of the purine salvage pathway is widely distributed in tissues and relatively high levels are found in the villi of epithelial cells lining the duodenum. Many studies have demonstrated alterations of ADA activity in the tumor tissue and serum in patients with lung, head and neck, breast and ovarian cancer [3 - 4]. In colon carcinoma [9] and in colorectal cancer [10], ADA was found to be elevated in the cancerous large bowel tissue. Studies done in gastric tissues in patients with gastric cancer have shown increased ADA activity in the cancerous tissues [11, 12]. But to the best of our knowledge, we could not get any report on serum ADA activity in gastric cancer.

The aim of the present study was to define the role of ADA as a tumor marker in gastric cancer, to assess its individual diagnostic accuracy in comparison with the conventional CEA and also to analyze the diagnostic relevance with the combination of both markers.

MATERIALS AND METHODS

The case-control study comprised of 22 patients with gastric cancer and 30 healthy individuals as control group. Newly diagnosed patients with gastric cancer who were not treated with any type of therapy were recruited from the Medical Oncology department of Sri Venkateswara Institute Of Medical Sciences (Tirupati, India) from 2003-2004. The control group was recruited from the people attending the master health checkup program of the hospital and staff belonging to Biochemistry clinical laboratory of the Institute during the same period and none of them had any type of cancer at that time or previously. Smokers and alcoholics and those with diabetes, hypertension, liver diseases, renal failure and active infection were excluded from the study. All the members were recruited with informed consent.

Sample Collection: A 5 ml venous blood sample from patients and controls was collected in plain tubes and centrifuged at 2,500rpm for 15 minutes at room temperature. Serum obtained was stored at -80°C until analysis.

Marker Determination: ADA was estimated by the colorimetric method of Galanti and Giusti using ADA–MTB Microxpress, Tulip Diagnostics (P) Ltd kit. CEA was estimated by quantitative solid phase enzyme linked immunosorbent assay, using UBI Magiwell, United biotech inc kit.

Statistical Analysis: The results were expressed as mean ± SD. Difference between parameters for control and patient group was assessed using unpaired student’s ‘t’ test and p < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed to study the diagnostic accuracy of the parameters as tumor markers. Their independent diagnostic relevance was assessed by performing Logistic regression analysis with the various cut off values. Correlation between the markers was assessed with the Spearman Rank test. Statistical analysis was performed using Microsoft excel spread sheets and SPSS for windows version 11.5.

RESULTS AND DISCUSSIONS

Result: Serum ADA and CEA levels were found to be significantly higher in the patient group as shown in table1. ROC curve analysis revealed statistically significant areas under the curve (AUC) for both, with ADA having more AUC when compared to CEA. When both markers were combined, AUC was found to be higher, when compared to that obtained individually as shown in table 2.

Applying the various cut off values for ADA and CEA in logistic regression model, ADA was found to have a higher odds ratio, with a higher outcome predictor variable with the ROC curve identified cut off value when compared to CEA. Combination of both ADA and CEA also gave a higher odds ratio when compared to that of CEA alone, as shown in table3.

Spearman’s rank correlation analysis between ADA and CEA revealed a highly significant correlation with r 0.440 (p<0.01).
Discussion: For gastric cancer large scale screening programs are not being performed due to the invasiveness of the diagnostic gastroscopy procedure with the added problem of the low detection rate of early gastric cancer by endoscopic means. Another attributing factor is the lack of a suitable alternative test which is noninvasive, simple to perform and is cost effective. CEA, one of the most widely used tumor marker in gastric cancer is currently being used for its prognostic value in the post operative follow up, which may be helpful in the early detection of recurrence. CEA is found to have a sensitivity of 20% in the early stage and 40-50% in the advanced stage, an indication of the lack of significant elevation in the early stages. In cancer there is an increased turnover of malignant cells and an associated increase in nucleotide metabolism leading to an increase in purine metabolizing enzymes. ADA is particularly sensitive to stimulation by growth factors and cytokines during rapid tissue proliferation. Numerous studies have documented an increase of ADA in very rapidly growing malignancies, where it has been documented as a tumor marker, while slow growing well-differentiated tumors do not express pronounced ADA activity. In the present study serum ADA was found to be significantly elevated in gastric cancer patients. The individual diagnostic accuracy of ADA as tumor marker was assessed by ROC curves which showed a more significant AUC for ADA, indicating ADA to have a better diagnostic utility than CEA. Cut off values with the best combination of sensitivity and specificity obtained from the ROC curves were 19.5IU/L for ADA with 82% sensitivity and 90% specificity and 6.4ng/ml for CEA with 85% sensitivity and 81% specificity. When both were combined and ROC curve was constructed the AUC was found to be more than that obtained individually for ADA and CEA, which signifies the increase in diagnostic accuracy with the combination of the markers. When both ADA and CEA were introduced into the logistic regression model as covariates, it was found that ADA had a higher Wald statistic (12.682 for ADA as compared to 6.976 for CEA) which shows the importance of ADA as a predictor variable. Similarly when the ROC curve identified cut off was applied in the logistic regression model as categorical variables, ADA had a higher Wald statistic (20.268 for ADA as compared to 14.583 for CEA). The predictor outcome obtained by ADA ROC curve cut off was given by the combination of both ADA and CEA applied as continuous variables (86.5%). Among them the contribution of ADA was more as evidenced by a
higher wald statistic (8.643 for ADA as compared to 3.377 for CEA). With a well identified ROC cut off value ADA was found to perform better than CEA as a tumor marker. When combination of both of these markers was used, the performance was similarly found to be better than that of CEA alone. This signifies that ADA has a pronounced role in gastric cancer, either used alone or in combination with CEA. Studies have shown significant ADA activity in various cancerous tissues like breast, bladder, kidney, colon including gastric tissue, which may point to the source of ADA, as being secreted from the tissues and its consequent elevation in the blood. These results probably reflect the changes in purine metabolism due to increase in DNA turnover in the cancerous tissues. Acceleration of the salvage pathway provides a selective advantage to cancerous cells to grow and develop more rapidly.

The simplicity of measuring ADA activity combined with its cost effectiveness gives an added advantage to consider ADA as a tumor marker in gastric cancer. ADA independently has a stronger predictor outcome than that of CEA as found in this study, which gives strength to support the inclusion of ADA as a tumor marker in gastric cancer. Further studies on these lines in a larger number of patients are needed to evaluate the role of ADA as a tumor marker, in the early detection of gastric cancer which remains the goal of identifying a tumor marker which can be included in the diagnostic panel.

REFERENCES
