

## **Evaluation of Plasma Malondialdehyde as a Biomarker in Patients with Carcinoma of stomach**

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### **Abstract**

**Carcinoma of stomach is the second most fatal malignancy. Tumor markers like carcinoembryonic antigen (CEA) are of little benefit in early diagnosis and monitoring the progress in these patients due to their low specificity and sensitivity. Hence there is a need to look for better biochemical markers. We conducted a case control study involving thirty healthy individuals and twenty two patients with stomach carcinoma. All of the control group members had normal blood chemistry, ECG, Chest X-ray, blood counts apart from a normal clinical examination. All the patients included in the study were untreated cases and had a confirmed histological diagnosis. The parameters studied included Malondialdehyde (MDA), and Carcinoembryonic antigen [CEA]. The mean age for patients and controls was 51 and 45 years, respectively. There was an increase in the levels CEA ( $p < 0.0001$ ) and MDA ( $p < 0.0001$ ) whereas the levels of vitamin E decreased ( $p < 0.05$ ) in the patient group compared to controls. The ROC analysis showed significant diagnostic accuracy of MDA as tumor marker. Logistic regression studies revealed a significant association of MDA with carcinoma stomach. As a biomarker for carcinoma stomach, MDA had significant diagnostic value independent of CEA levels. The sensitivity and specificity of MDA was more than that of CEA. Combined use of MDA & CEA was found to yield better diagnostic information than individual use of CEA**

**Key words:** Carcinoma stomach, Malondialdehyde, Biomarker.

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### **Introduction**

Carcinoma of stomach is the second most fatal malignancy in the world and is the cause of more than 7,50,000 deaths annually [1]. The high mortality rate from carcinoma of stomach arises from its late detection and surgical resection at an advanced stage of the disease [2]. Efforts directing at prevention, early detection and intensive therapy will go a long way in saving these patients. CEA was identified as early as 1969 in the sera of patients with large bowel cancer [3]. This discovery led to further work on identification of other tumor associated antigens (TAA) that could be useful for the early detection of cancer.

Tumor markers should be specific and sensitive to a given cancer type for early diagnosis. The markers studied till now include enzymes, hormones, antigens and proteins that are present in higher concentration in blood, other body fluids or tissues from cancer patients and proportional to the tumor burden but are not found in the non-tumor bearing hosts.[4]. Unfortunately, different markers produced by different tumors are neither specific nor sen-

sitive enough for screening as well as monitoring the prognosis. CEA, a marker elevated in the variety of cancers is not recommended for screening because of the false-positive results associated with benign and false-negative results due to CEA-nonproducing tumors [5]. Its sensitivity is only 30% to 40% for early stage tumors of the colon [6] and only 17% for other gastrointestinal malignancies [7]. Other tumor markers like carbohydrate antigen 72-4 (CA 72-4) and carbohydrate antigen 19-9 (CA19-9) have been evaluated for gastric cancer, but with little positive outcome [8].

Therefore, the scope for identification of newer markers opened up other molecular interactions like oxidative damage, immune mechanisms, and cytokines for clinical use in the management of malignancies either individually or in combination with the existing ones. Also, as most of the tumour markers lack in specificity, using a combination of markers always has a chance to increase the diagnostic ability of the tumour markers.

Reactive oxygen species [ROS] have been known to play an important role in the initiation and promotion of carcinogene-

sis and have been implicated in carcinogenesis in human as well as animal models [9]. They have been shown to be associated with the different steps of carcinogenesis either through structural DNA damage or interaction with oncogenes or tumor suppressor genes or immunological mechanisms [10]. MDA, the end product of lipid peroxidation, owing to its high cytotoxicity has been suggested to act as a tumor promoter and a co-carcinogenic agent [11]. Free radical intermediates of xenobiotic chemicals as well as oxygen radical production by chemical carcinogens have been related to environmental carcinogenesis [12, 13]. Infection with *H. pylori* has been shown to be a major predisposing factor for the development of malignancies in stomach and oxidative stress has been demonstrated in gastritis [1]. Also, oxidative stress has been shown to occur throughout the clinical history of any malignancy. The oxidative stress during the progression of disease is believed to be due to altered metabolic pathways in tumor cells, macrophageal intrusion of tumor tissue, and therapeutic interventions. Ionizing radiation yields a variety of reactive products, including free radicals that can damage macromolecules, including DNA [14]. It has also been shown that oxidative stress will come down with reduction in tumor mass after treatment [15]. An increase in serum/plasma MDA concentrations has been widely reported in various cancers [1, 16-21]. The increase in MDA levels were found even in early stages of cancer [17]. Increased amounts of lipid peroxidation end products have been demonstrated in tumor tissue itself [19-22] clearly pointing to the source of increased MDA levels in cancer patients.

Recently plasma MDA levels have been tested and found to be useful as tumor marker with predictive values as good as established tumor markers in gastrointestinal malignancies [22, 23]. However, to our knowledge there are no such studies on carcinoma of the stomach patients. Therefore, a case control study was undertaken in an attempt to establish the clinical use of plasma MDA as biomarker either independently or in combination with CEA in patients with stomach carcinoma.

## Material and Methods

Twenty two patients with Carcinoma of stomach attending medical oncology out patient clinic of S.V. Institute of Medical Sciences, Tirupati were recruited into the study along with 30 healthy individuals who served as controls. The control group was recruited from the people attending the master health checkup program of the hospital and staff of the department. All of them had normal blood chemistry, ECG, Chest X-ray, blood counts apart from a normal clinical examination. All the patients included in the study were untreated cases and had a confirmed histological diagnosis. None of patients and controls was smokers or alcoholics at the time of study. Persons with diabetes and or hypertension, renal failure and active infection were not included in the study. All the members were recruited with informed consent. The patient group consisted of 15 males and 7 females

with mean age of  $51 \pm 12$  years. The control group consisted of 18 males and 12 females with mean age of  $45 \pm 13$  years. The two groups were found to be matching with respect to age.

Five ml of heparinised blood was collected from patients and controls. Plasma was separated and analyzed immediately or stored at  $-80^{\circ}\text{C}$  until further analysis. Vitamin E was analyzed using HPLC [24]. Plasma MDA was estimated spectrophotometrically as thiobarbituric acid reactive substances (TBARS) [25]. CEA was estimated by ELISA using commercial kits (United Biotech Inc, USA). Urea levels were estimated by photometric method using commercial kits on Beckman CX9 Random access clinical chemistry analyzer.

The data analysis included comparison between groups, correlation and association studies. Mann Whitney U test was used to compare the differences in parameters between the patient and control groups. Spearman's Rank correlation was used to assess the association between parameters in the patient group. A scatter plot was plotted for MDA levels in the patients and controls. Receiver-operating characteristic (ROC) curves were constructed for these parameters and the areas under the curve (AUC) values were determined.

A p value of  $< 0.05$  was considered to be significant. The logistic regression model was established with the parameters as predictor variables and cancer status (the individual having cancer or not) as binary outcome variable. Analysis was performed using Microsoft excel spread sheets, SPSS for Windows Version 11.5.

## Results

The mean  $\pm$  S.E. of various parameters studied in patients and controls along with the significance of difference between the two groups are presented in Table - I. There was an increase in the levels of urea, CEA and MDA whereas the levels of vitamin E decreased in the patient group compared to controls. As shown in Figure: 1 the scatter plot shows the distinct distribution of MDA levels in the patients and controls. The patient group had visibly higher values compared to the control group. The Receiver operating characteristic (ROC) analysis was performed to test the diagnostic accuracy of MDA as tumor marker (Fig.2). It gave an area under the curve of 0.868 which was statistically significant. The predictive power of CEA and MDA as tumor markers was assessed with logistic regression by entering the parameters both individually and combined together. The cut off value for plasma MDA was obtained from ROC curves with the maximum sensitivity and specificity. The upper normal limit provided by the manufacturer was used as cut off for CEA. The odds ratios were significant for both MDA and CEA when introduced into the test either individually or together (Table 2).

Similarly, the diagnostic relevance of CEA and MDA at these cut off values was assessed in terms of their sensitivity and specificity, Positive predictive value (PPV) and Negative predictive value (NPV) (Fig.3). The logistic regression analysis showed that both CEA and MDA were comparable as predictors of disease. Individual use of MDA was found to be more diagnostic with the best combination of sensitivity, specificity, PPV and NPV. Combined use of CEA and MDA was also found to be useful as diagnostic markers for carcinoma stomach. The results of the study point to the possibility of using plasma MDA levels as an additional tumour marker.

**Table 1.** Showing the mean  $\pm$ SE. of various parameters studied in patients and controls

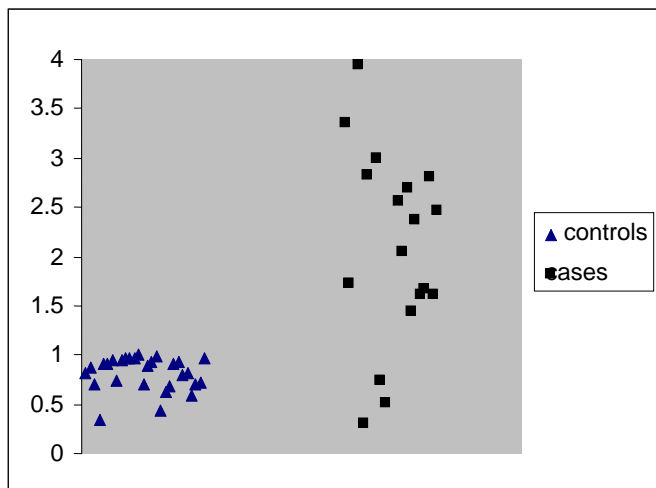
Parameter	Patients Mean $\pm$ SE	Controls Mean $\pm$ SE	P value
Urea (mg/dl)	28.27 $\pm$ 2.24	21.43 $\pm$ 1.04	0.014*
Carcinoembryonic antigen (ng/ml)	104.93 $\pm$ 38.99	3.83 $\pm$ 0.95	0.000*
Malondialdehyde (umol/L)	2.58 $\pm$ 0.40	0.81 $\pm$ 0.03	0.000*
Vitamin E (mg/L)	5.0 $\pm$ 1.48	8.81 $\pm$ 1.47	0.040*

\*Statistically significant

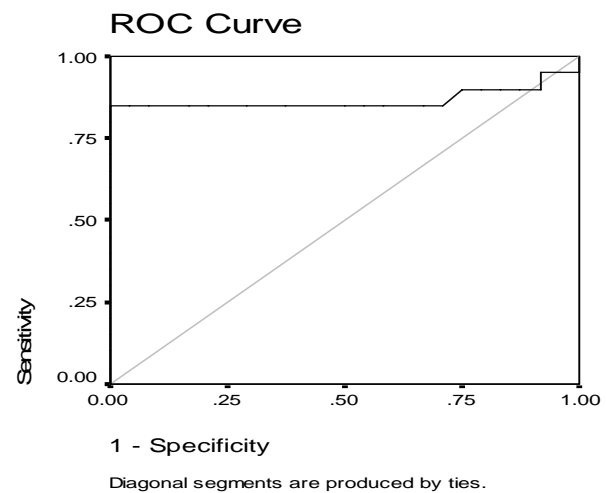
**Table 2.** Showing the logistic regression analysis for CEA and MDA individually and in combination.

Marker	CEA	MDA	MDA	CEA &MDA	B- Re- gre ssio n co- effi cie
Cutoff value	5ng/mL*	0.81 $\mu$ mol/L <sup>†</sup>	0.99 $\mu$ mol/L <sup>#</sup>	5.0 & 0.99* <sup>#</sup>	
B $\pm$ SE	3.12 $\pm$ 0.80	1.60 $\pm$ 0.72	5.10 $\pm$ 1.19	4.28 $\pm$ 1.14	
Odds ratio	22.667	4.958	164.333	72.800	
95% CI	4.71-109.01	1.19-20.54	15.81-1707.7	7.70-687.63	
P Value	<0.001	0.027	<0.001	<0.001	
Sensitivity (%)	85	85	85	72.2	
Specificity (%)	80	43.3	96.6	96.5	

nt, \*(Reference Mean), <sup>†</sup>(Control Mean), <sup>#</sup>(ROC Cutoff), \*<sup>#</sup>(CEA Reference Mean & MDA ROC Mean)

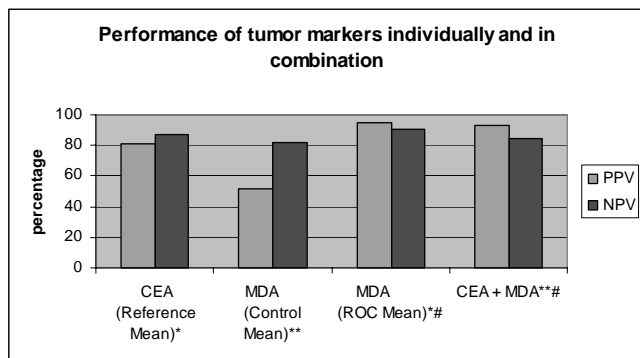


**Figure1.** Scatter plot for MDA in the patients and controls curve for MDA



**Figure 2:** Showing the receiver operating characteristic

Marker	AUC	P value	95% CI
MDA	0.868	0.000	0.727-1.008



**Figure 3.** showing the positive predictive value (PPV) and negative predictive values (NPV) for MDA and CEA individually and in combination

## Discussion

Carcinoma of stomach is a multifactorial process. Factors such as infection with *H. pylori*, cigarette smoking and chronic gastritis have been implicated in the pathogenesis. Of these *H. pylori* is believed to be a major etiological agent that causes chronic gastritis [1]. One characteristic event in gastritis is an infiltration of the sub-epithelial gastric lamina propria by phagocytes, mainly neutrophils and macrophages which produce large amounts of reactive oxygen species. These ROS have been shown to cause proteome changes in the gastric mucosa and DNA damage [26]. CEA has been shown to be a membrane constituent [27]. From the cell surface, CEA may be released into the interstitial spaces and thence into the circulation of the patient. CEA levels in serum have proven to be of little use in the early diagnosis of gastric cancer.

In the present study levels of MDA were found to be significantly elevated in patients with carcinoma of stomach ( $P=0.027$ ) as depicted in Fig: 1. This finding is in line with previous reports [1, 28, 29]. The renal and liver function tests were evaluated and found to be normal pointing to the absence of a source of ROS from these organs. The mild increase in urea levels ( $P<0.01$ ) in patients might as well be due to pre-renal causes like dehydration.

The antioxidant defense in plasma mainly lies with chain breaking antioxidant vitamins i.e. A, C and E. Vitamin E being lipid soluble and associated with lipoprotein fraction of plasma will be the one which is more likely to be involved first. In the present study, there was a significant decrease in the vitamin E levels corrected for cholesterol ( $P<0.05$ ) in the patient group pointing towards consumption of vitamin E. Vitamin E levels corrected for cholesterol take care of the changes in the lipid profile and confirm its decrease in plasma as a result of oxidative stress. Increased plasma MDA levels along with decreased antioxidant defense in the form of decreased Vitamin E levels and lack of evidence of other sources of oxygen radical production put together point towards continuous oxidative stress in stomach cancer patients. Our findings are supported by the reports that

showed that serum lipid peroxides observed in carcinoma stomach patients originate in the cancer tissue [29, 30].

The diagnostic accuracy of MDA as a biomarker was assessed by receiver operating characteristic (ROC) analysis which showed statistically significant area under the curve (Fig.2). The diagnostic relevance was then assessed using logistic regression analysis, Sensitivity, and specificity (Table-II), and the positive predictive value and negative predictive value analysis (Fig.2). The odds ratio was much higher (164.33) when the cut off value obtained from ROC curve was used when compared to the mean of the control group (4.958). This indicates that a properly identified cut off value will be very much useful as a diagnostic tool in stomach carcinoma. Combinations of markers have been studied previously by various authors in carcinoma of stomach, both in the serum [30] as well as in gastric juice [31]. These combinations apart from being of diagnostic and prognostic significance also increase the sensitivity compared to their individual use [32, 33]. Though there was a decrease in sensitivity (85% vs 72.2%) with use of combination of markers, there was an improvement in the specificity (80% vs 96.5%) and PPV (80.9% vs 92.8%) Spearman rank correlation test did not reveal any correlation between the CEA and MDA. This could possibly be due to different mechanisms of the production of these markers which further supports the use of these two markers in combination.

High mortality rate from carcinoma of stomach arises from its late detection. At present, early detection of carcinoma of stomach is difficult without interventional procedures. The proportion of cancers diagnosed as early gastric carcinoma clearly depending on the discovery of the sensitive diagnostic markers useful in screening. This naturally directs medical research on identification of sensitive markers that can be used for early detection. In light of no currently recommended markers for the early diagnosis of carcinoma of the stomach, our positive result points towards the presence of other markers which indeed can be used as markers for early diagnosis. Also, as our understanding of oxidative stress with respect to cause and consequence of disease increases, the efforts to make use of oxidative stress as biomarker will be the natural flow of events in medical research.

In conclusion, the elevated levels of MDA in stomach carcinoma could be used as an important parameter in patients at risk for this disease mainly due to its dual role as a mutagen and a tumor promoter. MDA used alone or in combination with CEA was found to provide significant diagnostic information for the diagnosis of carcinoma of the stomach. Our results suggest that combined use of these two markers can increase the diagnostic accuracy as against their individual use for diagnosis of the stomach carcinoma.

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