Online Journal of Health and Allied Sciences
Peer Reviewed, Open Access, Free Online Journal
Published Quarterly : Mangalore, South India : ISSN 0972-5997
Volume 8, Issue 4; Oct - Dec 2009

Original Article:

Physiological antioxidant system and oxidative stress in stomach cancer patients with normal renal and hepatic function

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Citation: Reddy EP, Reddy VS, Chandra Mouli K, Rao PVLNS. Physiological antioxidant system and oxidative stress in stomach cancer patients with normal renal and hepatic function. *Online J Health Allied Scs.* 2009;8(4):9
URL: http://www.ojhas.org/issue32/2009-4-9.htm
Open Access Archives: http://cogprints.org/view/subjects/OJHAS.html and http://openmed.nic.in/view/subjects/ojhas.html

Submitted: Oct 8, 2009; Accepted: Feb 11, 2010; Published: Apr 30, 2010

Abstract:
Role of free radicals has been proposed in the pathogenesis of many diseases. Gastric cancer is a common disease worldwide, and leading cause of cancer death in India. Severe oxidative stress produces reactive oxygen species (ROS) and induces uncontrolled lipid peroxidation. Albumin, uric acid (UA) and Bilirubin are important physiological antioxidants. We aimed to evaluate and assess the role of oxidative stress (OS) and physiological antioxidant system in stomach cancer patients. Lipid peroxidation measured as plasma Thio Barbituric Acid Reactive substances (TBARS), was found to be elevated significantly (p=0.001) in stomach cancer compared to controls along with a decrease in plasma physiological antioxidant system. The documented results were due to increased lipid peroxidation and involvement of physiological antioxidants in scavenging free radicals but not because of impaired hepatic and renal functions.
Key Words: Oxidative stress, Lipid peroxidation, Antioxidants, Stomach cancer.

Introduction:

Role of free radicals has been proposed in the pathogenesis of many diseases involving different organs such as breast, gastric, colon, multiple myeloma, ovarian and oral cancer.(1) Gastric cancer is a common disease worldwide and also one of the leading causes of cancer death (5th in male and 6th in female) in India. The estimated number of new cases each year is expected to rise from 10 million in 2000 to 15 million by 2020. Stomach cancer is the second most frequent cancer in the world.(2) The incidence of gastric cancer is different throughout the world and 60% of deaths from gastric cancer occur in developing countries.(3) Severe OS produces ROS and induces uncontrolled lipid peroxidation. Following lipid peroxidation aldehyde products, such as free fatty acids, malondialdehyde (MDA), occur and those products are referred to as TBARS. Since the cell membranes consist primarily of lipids, uncontrolled lipid peroxidation can cause cell injury and death. Considerable evidences have linked oxidative damage and cancer.

Lipid peroxidation is a normal phenomenon that occurs continuously at low levels in every individual. Those peroxidation reactions are toxic to cells and cell membranes; however, they are normally controlled by countervailing biological mechanisms. Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. Cells have developed a comprehensive array of antioxidants that act co-operatively *in vivo* to combat the deleterious effects of free radicals. Albumin, UA and bile pigments such as Bilirubin and biliverdin have also been proposed as important physiological antioxidants.(4) Roche *et al.,* recently reviewed the antioxidant properties of serum albumin.(5) The present study was planned to evaluate oxidative stress related lipid peroxidation marker MDA as TBARS and Physiological antioxidants in stomach cancer patients with normal kidney and liver function. Further, we aimed to assess the role of OS and physiological antioxidant system in stomach cancer group, compared to control group.

Patients and Methods:

Patients

After obtaining informed consent, 25 cancer patients attending to medical oncology unit, out patient department of SVIMS, Tirupati were recruited into the study along with 30 (18m and 12f) healthy controls. All the patients had a confirmed histological diagnosis and were fresh, untreated cases. Among 25 patients, 20 patients were presented with stomach cancer, 2 patients with rectum cancer and one each with foot, lung and breast cancer. As the number was disproportionate for various cancer types, we decided to include only 20 (15m and 5f) patients with stomach cancer to strengthen the study outcome. The members in control group were recruited from the healthy persons attending master health checkup Programme of the hospital and departmental staff. Healthy subjects and patients were age matched with median age of 45 and 51 years respectively. Exclusion criteria include active infection, renal failure, diabetes, and hypertension. None of the patient or control group participants were alcoholic or smokers.

Blood collection and methods

After 12 hour overnight fasting, heparinised venous blood was collected. Plasma was separated either to analyze immediately or to store at -80^{0}C until further analysis. CEA was estimated by ELISA using commercial kits (United Biotech Inc, USA). Urea, Creatinine, and ALT were estimated by photometric methods using commercial kits on Beckman CX9 Random access clinical chemistry analyzer. Lipid peroxidation marker,

MDA levels were evaluated spectrophotometrically as TBARS.(6)

Statistical analysis

All analyses were performed by using SPSS Statistical Analysis software for Windows Version 11.5. All data are expressed as mean ± standard error of mean. To find out the Differences in plasma concentrations of different variables between groups, Mann Whitney U test was used. *P*-values of less than .05 were considered to be significant.

Results:

Carcino embryogenic antigen (CEA) was observed to be higher in stomach cancer patients as compared to the controls (*P*=0.001). Table 1 summarizes the changes in parameters studied between patient and control groups.

Table 1: The biochemistries studied in stomach cancer group and their comparison to healthy controls

Variable	**Control Group** Median (Mean ± SE)	**Stomach Cancer** Median (Mean ± SE)	**P value**
Urea (mg/dL)	22.0 (21.4±1.0)	28.5 (30.1±2.8)	0.004*
Creatinine (mg/dL)	0.7 (0.69±0.03)	0.85 (0.88±0.10)	0.23
ALT (IU/L)	18.0 (20.1±1.5)	13.0 (18.5±3.7)	0.11
CEA (ng/mL)	1.2 (3.8±0.95)	19.2 (80.3±28.1)	0.001*
MDA (µmol/L)	0.89 (0.81±0.03)	2.76 (6.51±2.66)	0.001*
Albumin(g/dL)	3.8 (3.7±0.06)	2.9(2.8±0.1)	0.001*
Uric acid (mg/dL)	4.3 (4.4±0.2)	3.4 (3.7±0.4)	0.052
Bilirubin(mg/dL)	0.6 (0.6±0.05)	0.5 (1.03±0.42)	0.35
(* Statistically significant)			

Renal and Liver function

Functional status of kidney was assessed by measuring urea and creatinine levels. Creatinine always accurately reflects renal function. In this study, we report a non-significant change in serum creatinine (p=0.23) between patient and control groups indicating that the renal function was not impaired in patient group. The mild increase in urea levels of patients might be due to pre-renal causes.

Liver function was assessed by evaluating serum UA (p=0.052), Bilirubin (p=0.35) and Alanine transaminase (ALT) (p=0.11) levels in both groups was found to be normal as we could observe no significant change in these parameters between groups.

Oxidative stress and physiological antioxidant response

Plasma lipid peroxidation measured as TBARS was observed to be significantly (p=0.001) elevated in stomach cancer group compared to healthy controls. The decrease (significant only for albumin, p=0.001) in all the three physiological antioxidants viz., Albumin, UA, and Bilirubin was observed in patient group compared to controls.

Discussion:

The findings of the study suggest that (1) OS in stomach cancer stimulates lipid peroxidation evidenced by the elevated levels of plasma TBARS, (2) Decreased plasma levels of physiological antioxidants were found in patient group with normal hepatic and renal function. The documented results were due to increased lipid peroxidation and involvement of physiological antioxidants in scavenging free radicals but not because of impaired hepatic and renal functions.

Lipid peroxidation

Oxygen radicals, produced in man during oxygen metabolism, are toxic to cell membranes, DNA, and RNA and may initiate carcinogenesis.(7) The process of lipid peroxidation is one of the oxidative conversions of polyunsaturated fatty acids to MDA, the main sensitive parameter of lipid peroxidation. Elevated plasma TBARS was reported in gastric (8,9) and other cancer types (10,11) as well.

In the present case-control study, we report an increase in lipid peroxidation marker MDA measured as TBARS in stomach cancer patients versus healthy controls. The difference was statistically found to be significant. Increased levels of TBARS in our study confirm increased oxidative stress in stomach cancer patients. Similarly, increased TBARS concentration in both plasma and erythrocytes of gastric cancer patients was reported recently from India by Pasupathy et al (12), who also found higher lipid peroxidation in smoking gastric cancer patients than non-smoking patients with gastric carcinoma. This marked increase in lipid peroxidation may be due to over production of Freeradicals, excessive generation of lipid peroxidation products in tumor tissue, and due to impaired antioxidant system that favor accumulation of free radicals. As renal failure itself is a source of OS, normal renal function observed in our study indicate that increased TBARS might be due to increased lipid peroxidation, not because of renal failure and decreased MDA clearance.

Physiological Antioxidants

Plasma Albumin

In general, albumin represents the major and predominant antioxidant in plasma, a body compartment known to be exposed to continuous OS. A large proportion of total serum antioxidant properties can be attributed to albumin. Previous works have shown that more than 70% of the free radical-trapping activity of serum was due to human serum albumin (HSA) (13), continuously exposed to OS.(14) The N-terminal DAHK sequence is known to inhibit LDL lipid peroxidation and superoxide dismutase-like activity of DAHK/Cu complex by significantly preventing the formation of ROS was already reported. Moreover, the reduced Cys34 of albumin has the ability to scavenge hydroxyl radicals.(15) Highly reactive species are able to induce oxidative degradation of protein *in vitro*.(16) Structural modification of albumin induced by glucose or free radicals impairs its antioxidant properties. Moreover, albumin is a negative acute phase protein synthesized by liver whose levels fall in response to infection, injury and neoplasia.(17)

The significant decrease in mean serum albumin levels in colon cancer was reported earlier by Ko et al.(4) Similarly in stomach cancer patients we demonstrate a decrease in plasma albumin levels compared to healthy controls. Significant decrease in plasma albumin levels observed in our study was due to its protective effects against oxidative attack. In addition, the nutritional status of patient group might partly explain the decreased plasma albumin levels.

Plasma Uric acid

UA is a powerful antioxidant quencher of singlet oxygen as potent as ascorbate and is potentially more important as an antioxidant in normal physiology.(7) Little is known about the role of UA for cancer. It has been hypothesized that the antioxidant properties of UA may play a crucial role in cancer etiology by preventing the formation of oxygen radicals, thereby protecting against carcinogenesis.(7,18)

Mazza et al found that UA could protect against cancer by influencing the toxic and carcinogenic effects of oxygen radicals.(19) Similarly, our study could demonstrate decrease in plasma UA levels in cancer patients than in healthy controls,

possibly supporting the antioxidant and protective effect of UA against OS in cancer patients. Ames et al., (7) proposed that the antioxidant properties of uric acid may act to prevent formation of oxygen radicals and thereby protect against carcinogenesis. In contrast, several studies reported that elevated UA levels as a risk factor for cancer mortality in general populations.(20,21) Recently, Bozkir et al (22) reported UA levels of lung cancer patients to be significantly lower than those of healthy controls. There have been reports showing markedly elevated levels of UA in cancer patients attributed to the malignant process resulting from the increased nucleic acid turnover in the rapidly proliferating diseased tissue.(23)

Despite of this controversy, renal dysfunction would affect the plasma UA levels. In our study patients presenting normal renal function, decrease in UA level does not reflect increased renal secretion as a result of tubular damage. Since there are no enzymes capable of degrading UA in man, the decrease in plasma of cancer patients must be ascribed to its action as an *in vivo* scavenger of oxidants or degradation by radical and oxidant producing enzymes. Further, serum UA levels might also be influenced by endogenous production and diet.(24)

Plasma Bilirubin

Bilirubin in its free form and when bound to its physiological carrier protein of extracellular fluids, albumin, efficiently scavenges radicals.(25) An indirect antioxidant activity of albumin comes from its ability to transport bilirubin, which binds with high affinity to the molecule at Lys240.(26) Such albumin-bound Bilirubin was shown to act as an inhibitor of lipid peroxidation.(27) Wei et al showed an inverse relationship between risk of cancer development and serum bilirubin levels.(28) We observed a decrease but statistically insignificant in fasting plasma bilirubin levels of stomach cancer patients than controls. The difference was not statistically significant, presumably because of the low number of patients involved in the study, which is in line with the observations reported by Ko et al in colon cancer.(4) Our findings in stomach cancer patients indicate that bilirubin exhibits potent antioxidant properties in counteracting oxidative stress and may contribute to protection against oxidative stress-mediated cancer disease.

In summary, the increase in oxidative stress in stomach carcinoma was evidenced by significant rise in plasma lipid peroxidation marker MDA measured as TBARS. It is interesting to observe that there was a significant fall in serum albumin level in patients due to its protective effect against deleterious oxidative damage. It is also interesting to observe that uric acid and Bilirubin were lowered in these patients, which is another means of highlighting their protective action in the better control of oxidative stress. Although the difference between patients and controls was not statistically significant for UA and Bilirubin, significant difference might be achieved if larger studies were conducted. The documented results were due to increased lipid peroxidation and involvement of physiological antioxidants in scavenging free radicals but not because of impaired hepatic and renal functions. Our study ascertains the importance of monitoring and controlling OS in carcinoma of stomach.

Acknowledgements:

We sincerely acknowledge the help and support of Dr. K. Sambasivaiah, Assoc. Prof. of Oncolgy, in providing samples and clinical material.

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