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SHORT REPORT

Effect of chronic smoking on lipid peroxidation and antioxidant status in gastric carcinoma patients

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Abstract

Oxidative stress plays an important role in malignant transformation and is postulated to be associated with increased lipid peroxidation. We determined the effects of chronic cigarette smoking on lipid peroxidation and antioxidant status in 100 male patients with gastric cancer and an equal number of age-matched healthy control subjects. The mean (SD) level of thiobarbituric acid reactive substances (TBARS) was higher in plasma (healthy non-smokers 3.1 [0.2]; healthy smokers 4.6 [0.2]; gastric cancer non-smokers 6.5 [1.0]; gastric cancer smokers 8.9 [3.1]) and erythrocytes (3.3 [0.6]; 4.6 [0.1]; 8.3 [0.9]; 13.2 [5.1]) from gastric cancer patients when compared with control subjects. TBARS level was higher in smokers than non-smoking gastric cancer patients. The activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-treansferase, reduced glutathione, and vitamins A, E and C were decreased in gastric cancer patients who were smokers as compared to other groups ($p<0.001$). Thus, there occurs lipid peroxidation and possible breakdown of antioxidant status in cigarette smoking, which may increase the risk of gastric cancer.

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Introduction

Cigarette smoke is known to contain a large number of oxidants, which are capable of causing an increase in the generation of various reactive oxygen species (ROS) like superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl ($OH^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals. These ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation. Tobacco smoke contains a variety of carcinogens including *N*-nitroso compounds and nitrogen oxides that may promote endogenous formation of *N*-nitroso compounds, which have been linked to gastric carcinogenesis.[1]

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. Low levels of enzymatic and non-enzymatic antioxidants in circulation have been found to be associated with an increased risk of cancer. We determined the effects of chronic smoking on the extent of lipid peroxidation and the status of antioxidants in gastric cancer patients.

Methods

One hundred men with gastric cancer and 100 age-matched healthy men were evaluated from January 2005–July 2007. Gastric cancers were diagnosed by histology of gastric biopsy samples. Patients or healthy subjects who smoked were included if they smoked >25 cigarettes per day for minimum 2 years. Patients who were receiving chemotherapy or radiotherapy at the time of the study, those with *H. pylori* infection, and other co-morbid illnesses that might affect free radical status were excluded. Controls were free of any medication for at least one week before and during the study. The study was approved by the Bioethics Committee of K.G. Hospital, and informed verbal consent was obtained from all subjects.

Blood samples were collected and the plasma was separated by centrifugation at 1000×*g* for 15 minutes. After centrifugation, the buffy coat was removed and packed cells

were washed three times with physiological saline. 500 µL of erythrocytes was lysed with hypotonic phosphate buffer (pH 7.4). The hemolysate was separated by centrifugation at 2500×*g* for 15 minutes at 2°C.

Lipid peroxides were estimated by measurement of TBARS in plasma[2] and in erythrocytes[3] by previously described methods. Levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activity, glutathione-*S*-treansferase (GST) activity, and reduced glutathione (GSH) in plasma were determined.[4–8] Erythrocyte GSH content was determined with dithionitrobenzoic acid.[9] Plasma vitamins (A, E and C) were estimated by standard methods.

Data were expressed as mean (SD) and analyzed using one-way analysis of variance (ANOVA) using SPSS version 10 (*North Carolina*, USA). Duncan's multiple range test (DMRT) was used for individual comparisons. A p value of ≤0.05 was considered as significant.

Results

Table 1 shows demographic characteristics and biochemical variables in healthy control and patients with gastric cancer subjects. TBARS level was higher in both plasma and erythrocytes in patients with gastric cancer compared with that in healthy subjects (p<0.001). Amongst gastric cancer patients, the concentration of TBARS was higher in smokers. The levels of enzymatic (SOD, CAT, GPx and GST) and non-enzymatic (vitamins A, E and C) antioxidants in gastric cancer subjects were lower than those in healthy controls (p<0.001).

Discussion

We found increased TBARS concentration in plasma and erythrocytes from patients with gastric cancer, indicating that there is a non-specific over-production of free radicals in these patients. The extent of plasma lipid peroxidation was higher in gastric cancer patients who smoked as compared to those who did not.

The over-production of free radicals may be due to excessive generation of lipid peroxidation products in tumor tissue, and their subsequent release into circulation. Patients with gastric cancer may have increased lipid peroxidation due to a poor enzymatic and non-enzymatic antioxidant defense system. Tobacco smoke has been reported to stimulate H_2O_2 and hydroxyl radicals. Aromatic amines, nitrogen oxide, heavy metal ions like Cd^{2+} and other potential carcinogens found in smoke can induce lipid peroxidation. Nicotine has been demonstrated to inhibit apoptosis, thereby facilitating cancer development.[10]

Table 1. Demographic characteristics and biochemical variables in healthy controls and gastric cancer patients

	Control		Gastric cancer	
Parameter	Non-smokers	Smokers	Non-smokers	Smokers
Number of subjects	50	50	50	50
Mean age (years)	56 (12.4)	54 (14.5)	59 (11.7)*	53 (10.4)**
Body mass index (kg/m^2)	35 (9.7)	33 (10.2)	30 (9.8)	26 (7.8)‡
Alcohol (n [%])	–	1 (2)	1 (2)	1 (2)
Hypertension (n [%])	1 (2)	3 (7)	6 (12)	8 (16)
Hemoglobin (g/dL)	14.7 (1.6)	13.5 (1.2)	10.2 (1.2)*	8.5 (1.5)‡
Plasma TBARS (nmol/mL)	3.1 (0.2)	4.6 (0.2)†	6.5 (1.0)*	8.9 (3.1)‡**
Erythrocyte TBARS (pmol/mg Hb)	3.3 (0.6)	4.6 (0.1)†	8.26 (0.9)*	13.2 (5.1)‡**
Plasma GSH (mg/dL)	39.0 (1.7)	30.1 (3.5)†	27.1 (3.1)*	21.2 (3.5)‡**
Erythrocyte GSH (mg/dL)	56.3 (7.5)	42.9 (10.8)†	44.4 (6.7)*	27.5 (9.2)‡**
SOD (Unit^A/mg Hb)	3.7 (0.3)	2.6 (0.1)†	2.1 (0.1)*	1.9 (0.1)‡**
CAT (Unit^B/mg/Hb)	69.5 (6.6)	51.4 (9.0)†	43.7 (7.7)*	32.2 (6.0)‡**
GPx (Unit^C/mg/Hb)	9.3 (1.6)	6.9 (0.8)†	6.03 (0.7)*	3.74 (0.7)‡**
GST (Unit^D/mg/Hb)	2.3 (0.3)	1.8 (0.3)†	1.4 (0.3)*	0.8 (0.3)‡**
Vitamin A (mg/dL)	0.8 (0.1)	0.5 (0.1)†	0.4 (0.1)*	0.3 (0.1)‡**
Vitamin E (mg/dL)	1.2 (0.3)	0.8 (0.2)†	0.6 (0.1)*	0.4 (0.2)‡**
Vitamin C (mg/dL)	1.1 (0.2)	0.7 (0.2)†	0.5 (0.1)*	0.4 (0.1)‡**

Values are as mean (SD). p <0.017 was considered significant

‡Gastric cancer smokers vs control smokers; **Gastric cancer smokers vs gastric cancer non-smokers; *Gastric cancer non-smokers vs control non-smokers; †Control smokers vs control non-smokers

A– One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 minute; B – µmol of H_2O_2 consumed/minute; C – µg of GSH consumed/minute; D – µmol of CDNB – GSH conjugate formed/minute

SOD catalyzes dismutation reaction of the toxic $O_2^{\bullet-}$ to molecular oxygen and H_2O_2. Decreased activity of SOD has been reported in malignancies. Superoxide, a highly diffusible radical, can transverse membranes and cause deleterious effects at sites far from the tumor. It is possible that lipid peroxidation of erythrocytes in patients with gastric cancer is due to $O_2^{\bullet-}$ produced by the tumor as well as low activity of SOD within the red cells.[11]

Oxidative stress may cause changes in the glutathione redox state in cancer tissues. MDA may react with amino acid residues of proteins and lead to their oxidative modification; it can also increase oxidative stress by promoting cellular consumption of glutathione and by inactivating GPx. GSH can act directly as a free radical scavenger by neutralizing $HO^{\bullet}$, or indirectly by repairing initial damage to macromolecules inflicted by $HO^{\bullet}$. A decrease in GSH content in circulation has been reported in malignancies. The formation of GSSG during reduction of peroxides, or as a consequence of free radical scavenging, is potentially cytotoxic.[12] We found that had lower levels of activities of GPx and GST.

In patients with gastric cancer who smoked, the plasma level of vitamins was lower as compared to that in the other three groups, possibly due to increased turnover of the vitamin. It could also be due to its increased consumption during recycling of vitamin E or β-carotene that is oxidized in the course of scavenging free radicals and reactive oxidant species in cigarette smoke.[12]

In conclusion, chronic smoking enhances erythrocyte lipid peroxidation in gastric carcinoma patients with concomitant failure of both plasma and erythrocyte antioxidant defense mechanisms. The low antioxidant status of healthy smokers may predispose them to oxidant-mediated tissue damage, which may increase the risk of gastric cancer.

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News and notices

The 19th national conference of Pediatric Gastroenterology, Hepatology & Nutrition Subchapter of IAP will be held at Calicut, Kerala on October 10–11, 2009.

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