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Evaluation of Oxidative Stress, Antioxidant Status and Serum Vitamin C Levels in Cancer Patients

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Abstract Taking into account the importance role of lipid peroxidation and antioxidants in the prevention and incidence of cancer, the present study was carried out to determine oxidative stress, serum total antioxidant (TAS), and vitamin C levels in cancer patients. Malondialdehyde(MDA), total antioxidant status, and vitamin C levels of 57cancer patients aged 19–80 years and 22 healthy subjects (control group) aged 22–76 years were evaluated. Serum concentrations of MDA as thiobarbitaric acid complexes were measured by fluorometry method, the serum TAS by using commercial test kits from Randox Laboratories, and vitamin C by using spectrocolorimetric method. The mean serum MDA concentrations of all cancer groups except lung cancer were significantly higher than control group ($P<0.004$). The mean total antioxidant status was insignificantly higher than control group. The mean serum vitamin C level was significantly lower in patients as compared to the healthy subjects (PV<0.0001). In conclusion, an alteration in the lipid peroxidation with concomitant changes in antioxidant defense system in cancer patients may be due to excessive oxidative stress. Serum low levels of vitamin C in the different type of cancer patients in spite of adequate daily intake may be due to increased utilization to scavenge lipid peroxides as well as their sequestration by tumor cells.

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Introduction

Over the past three decades, extensive efforts were made to treat cancer. However, as recent statistics show, the incidence and mortality from cancer have, in general, not diminished but have instead increased [1, 2]. It seems that reactive oxygen species (ROS) are major molecules that are involved in the etiology of a variety of human disease and their role of on the initiation and progression of carcinogenesis through multiple mechanisms are recognized [3].

ROS generation is controlled by a large number of antioxidant systems that act as protection against ROS. These systems consist of antioxidant enzymes as well as non-enzymatic antioxidants such as ascorbic acid and alpha-tocopherol. Oxidative stress is defined as a disturbance in the equilibrium between ROS and detoxifying antioxidant systems; an excess of ROS leads to oxidative damage to cellular constituents such as lipid, protein, and DNA [4–6]. The interaction of ROS with DNA results in an impairment of the genetic material in the cell nucleus. In the absence of an adequate antioxidant system, accumulated DNA damages cause mutagenic alterations that result in aging, neurological disease, cancer, etc. [7–9].

Vitamin C is considered to be one of the most and prevalent antioxidative components of fruit and vegetable, and it could exert chemopreventive effects [10]. It has generally been acknowledged that vitamin C protects cells from oxidative DNA damage, thereby blocking the initiation of carcinogenesis [5].

In addition, blood vitamin C has been shown to be inversely correlated with biomarkers of oxidative stress even after adjusting for other antioxidants [11], suggesting that vitamin C may decrease oxidative stress.

Taking into account the importance role of lipid peroxidation and antioxidants in cancer patients, we carried out this study to evaluate the malondialdehyde (MDA) as a marker of lipid peroxidation and total antioxidant status (TAS) in patients with cancer. We also measured serum vitamin C levels of the patients to elucidate further the relation between vitamin C and cancer.

Subjects and Methods

In this study, we recruited 57 (39 men, 18 women) volunteer cancer patients aged 19–80 who referred to Radiotherapy department. Before radiotherapy planning, radiation oncologist registered patients with clinical variables cancer location and tumor size, nodes, and metastasis staging. Patients on the basis of location of tumor were classified to gastrointestinal, head–neck, and lung cancer groups. There were healthy volunteers (age- and sex-matched) serving as the control subjects, ranging in age from 22 to 76 years.

Methods

Blood samples (7 ml) were collected from fasting subjects via venipuncture to determine serum TAS and MDA and vitamin C levels. Serum were separated by centrifugation within 1 h. To deproteinize, 1 ml of metaphosphoric acid (6 g/dl) was added to 0.5 ml of serum sample for vitamin C measurement. All samples were stored at −70°C until analysis.

Chemical Analysis

Serum concentrations of MDA as thiobarbitaric acid complexes were measured by fluorometry method [12]. Commercial test kits from Randox Laboratories were also applied to measure the serum TAS.

For measurement of vitamin C, after melting of serum sample, 1 ml metaphosphoric acid (6 g/dl) was added. The precipitate was removed by centrifugation for 10 min (1,500×*g* at 4°C), and the supernatant fluid was assayed using the 2, 4-dinitrophenylhydoxazin spectrocolorimetric method [13].

Statistical Analysis

Descriptive statistics were obtained for all study variables for each study group. One-way analysis of variance comparing the four study groups (controls groups and gastrointestinal, head and neck, and lung cancer patients) for each variable was applied. Tukey's test, adjusted for multiple comparisons, was used for data analysis. All statistical analyses were two-tailed and a value of $P<0.05$ was considered statistically significant.

Results

The characteristics of patients and control group are shown in Table 1. There was no significant difference in body mass index (BMI) of patients among study groups ($P=0.310$). The results showed that the mean serum MDA concentrations in the serum of all cancer groups except patients with lung cancer were significantly higher than those of controls ($P<0.004$). The levels of serum TAS in all groups were higher than in control group, but the differences were not significant (Table 2); we did not observe any significant difference among different cancer groups. In general, the mean serum vitamin C levels of patients were significantly lower than control groups (PV<0.0001).

On the basis of the reference intervals for vitamin C, 80.7% ($n=46$) of patient were in deficiency (<0.2 mg/dl), 12.2% ($n=7$) in marginal deficiency (0.2–0.4 mg/dl), and 7% ($n=4$) in normal levels, while in control group, vitamin C level of all subjects were in normal level.

Discussion

It is well known that excess generation of oxygen-derived radicals can cause oxidative damage [14, 15]. The end product of lipid peroxidations, malondialdehyde, due to its high cytotoxicity and inhibitory action on protective enzymes, is suggested to act as a tumor

Table 1 Characteristics of Patients and Control Group

Study group	Number of subjects	Age (years)	Gender	Stage disease (*n*)	BMI (kg/m^2)
Gastrointestinal cancer	22	50–80	15 M, 7 F	III (18), IV (4)	22.2±1.0
Head and neck cancer	19	20–75	16 M, 3 F	III (11), IV (8)	22.7±0.9
Lung cancer	16	19–71	9 M, 7 F	III (10), IV (6)	22.3±1.2
Control	22	22–76	12 M, 10 F	–	23.4±0.7

Table 2 The Mean of Biochemical Profile in Studied Subjects

Study group	Vitamin C (mg/dl)	MDA (nmol/L)	TAS (mmol/L)
Gastrointestinal cancer	0.15±0.004*	8.54±1.83**	1.23±.054
Head and neck cancer	0.20±0.03*	6.92±1.24**	1.33±0.056***
Lung cancer	0.13±0.03*	4. 6±2.4	1.25±0.047
Total	0.17±0.02*	6.7±0.97**	1.25±0.25
Control	0.89±0.07	0.27±0.11	1.17±0.02

*P=0.000, **P<0.004, ***P<0.04 (significantly different from control)

promoter and a co-carcinogenic agent. An inverse relationship has been observed between lipid peroxidation and the rate of cell proliferation, with highly proliferating tumors showing low levels of lipid peroxidation [16]. Studies showed that in contrast to decreased lipid peroxidation in tumor tissues, enhanced lipid peroxidation was observed in the circulation of cancer patients [17].

The results of the present study showed that the levels of lipid peroxidation were significantly higher in serum of the all cancer groups as compared to that of healthy subjects. However, we observed a remarkable difference of MDA levels between patients with lung cancer and control group, but not significant, which may be due to the small sample size in this group.

Beevi and Rasheed [18] found that the MDA levels significantly elevated in oral cavity cancer when compared to control group. Manju et al. [17] observed a significant increase in the serum MDA concentration in patient with cervical cancer. Also, the results of several studies indicated that the lipid peroxidation levels in blood of different types of cancer patients were significantly enhanced as compared with normal subjects [19–21].

Our findings were in agreement with most of the earlier studies which suggested that there was a possibility of the accumulation of ROS which results in significantly higher lipid peroxidation at cellular and molecular levels.

Our data showed that there were significantly higher levels of serum total antioxidant status in the blood of patients with head and neck cancer than those of controls; however, the levels of serum total antioxidant in patients with gastrointestinal and lung cancer were insignificantly higher than in control groups. These results suggested that there may be upregulation of antioxidant enzymes induced by ROS in patients with cancers [4]. It appears that total antioxidant status in serum or plasma is tightly regulated [22].

In this study, serum vitamin C levels were significantly lower in all patients as compared with the healthy subjects, which may be due to increase oxidative stress in the patients.

Manju et al. [17] and Senthil et al. [16] observed that serum vitamin C in patients with cervical cancer were significantly lower than in control group. Yeh et al. [4] found that there was significant decreases in the levels of blood vitamin C of the patients with breast cancer compared to those of the control subjects. Also, Khanzode et al. [23] found that the mean concentration of serum vitamin C in gastric cancer patients was significantly lower than in control group.

Extracellular vitamin C may protect against oxidant and oxidant-mediated damage. In vitro studies suggest that vitamin C may be the primary antioxidant in plasma for aqueous peroxyl radical as well as lipid peroxidation product [24]. Although vitamin C has been known to stimulate immune function, inhibit nitrosamine formation, and block the metabolic activation of carcinogens, its cancer-preventive effects may be associated mainly with its protective effects against oxidative stress. Reactive oxygen intermediates (ROIs) are major molecules that can cause cancer through multiple mechanisms. The carcinogenic

effect of oxidative stress has been primarily focused on the genotoxicity of ROIs, and vitamin C can protect against oxidative DNA damage, which is implicated in tumor initiation. ROIs have also been known to play a significant role in the promotional stage of carcinogenesis [25].

In the present study, serum ascorbic acid concentration was lower in cancer patients as compared to the normal reference values, whereas concentration of serum MDA was higher than in control subjects. From the observation, it may be contended that serum vitamin C level decreases with resultant increase in lipid peroxidation. Possible explanation to our findings might be that more lipid peroxidation could induce more membrane damage, as a result of which, extracellular concentration of free radical may increase. Therefore, increased amount of ascorbic acid may be required to scavenge free radical which is probably reflected by low serum concentration in cancer patients.

In conclusion, an alteration in the lipid peroxidation with concomitant changes in antioxidant defense system in cancer patients may be due to excessive oxidative stress. Also, low levels of vitamin C in the different type of cancer patients may be due to increased utilization to scavenge lipid peroxides as well as their sequestration by tumor cells.

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