RESEARCH COMMUNICATION

# Effects of Cigarette Smoking on Lipid Peroxidation and Antioxidant Status in Cancer Patients from Western Nepal

**T Nagamma[1*], K Anjaneyulu[3], J Baxi[2], P Dayaram[1], PP Singh[4]**

## Abstract

**Objectives: There is growing evidence that oxidative stress (OS) has a causal relationship with cancer and a weak antioxidant defense can aggravate it further. We therefore, undertook this study to examine lipid peroxidation (TBARS), total antioxidant activity (TAA), ascorbic acid (vitamin C) and α- tocopherol levels in cancer patients, with special attention to the influence of smoking. Methods: The study subjects were 42 patients (61.19±10.1yrs) suffering from cancer and 43 normal subjects (NS) (56.69±19.1yrs). Plasma levels of TBARS, TAA, vitamin C and α- tocopherol were estimated. Results: TAA and α-tocopherol levels were significantly lower and TBARS levels significantly higher in cancer patients when compared to NS. In smoking cancer patient's α-tocopherol levels were significantly low and TBARS significantly raised. Conclusion: Our observations indicate that increased lipid peroxidation, reduced total antioxidant activity and α-tocopherol levels are associated with cancer development, with and without smoking. However, a greater reduction of TAA in smokers may be due to increased oxidants introduced by smoking.**

**Keywords:** Smoking - oxidative stress - antioxidants - cancer - Western Nepal

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## Introduction

Environment is an integral part of individual and population level risk factors, and cancer statistics reflect the sum total of environmental risk mediated by lifestyle choices, genetic inheritance, and metabolic factors. Smoking is one of the major lifestyle risk factors in cancer. Cigarette smoke is a complex mixture of over 7000 chemical compounds (Rodgmen and Perfetti, 2009). Each puff of cigarette smoke contains $10^{14-16}$ Reactive oxygen species (ROS) like superoxide ($O_2^{\bullet-}$), hydrogen peroxide ($H_2O_2$), hydroxyl ($OH^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals (Church and Pryor, 1985; Hecht, 2007). Smoke may enhance oxidative stress by following three reasons, first, tobacco is rich in pro-oxidants, which are further supplemented during smoking and chewing, second, these pro-oxidants consume more antioxidants and third, smokers have a tendency for low intake of dietary antioxidants.

There is evidence that smoke induced oxidative stress and lower plasma antioxidant concentration plays an important role in cancer development such as lung, larynx, esophagus, pancreas, prostate and bladder (Scheetman et al., 1989; Lykkesefeldt et al., 2000; Singh et al., 2004; Mahmood et al., 2007; Yanbaeva et al., 2007). Mutagens and carcinogens present in tobacco smoke are capable of initiating and promoting oxidative damage to DNA, mutations of oncogenes and altered gene expression (Taioli, 2008).

The aim of this study was to evaluate the effect of cigarette smoking on lipid per oxidation and antioxidant status in cancer patients from western region of Nepal.

## Materials and Methods

This study included a total of 42 patients suffering from cancer. Out of 40 cancer patients 20 were lung cancer patients, 13 were breast cancer, 7 were neck cancer and 2 were having GI cancer. 43 Normal subjects (NS) (56.69±19.1Yrs) without any known diseases serving as controls. This study was conducted in Manipal Teaching Hospital, Pokhara, Nepal. The mean age of patients and controls were 61.19±10.1and 56.69±19.1years respectively.

Each of the main groups subdivided into smokers and non smokers. The group of cancer patients included 30 smokers smoking >80 cigarettes per week with mean duration of smoking was equal to 28 ± 2 years and 12 non smokers are never smoked. NS included 26 smokers smoking <50 cigarettes per week with mean duration of

*[1]Department of Biochemistry, [2]Department of Surgery, Manipal College of Medical Sciences, Pokhara, Nepal, [3]Department of Anatomy, Sikkim Manipal Institute of Medical Sciences, India, [4]Department of Biochemistry, Era's Lucknow Medical College, India*
**For correspondence : nagu7890@yahoo.co.in*

smoking 25± 2 years and 18 non smokers were never smoked. Cancer patients who were on radiotherapy, antioxidant supplementation and any other illness were excluded from the study. None of the healthy volunteers were taking any drug/medication that would affect the oxidant and antioxidant level.

*Sample collection and Preparation*

Prior verbal consent was taken from all the participants. Six ml of blood was collected into EDTA bottles from each subject by venipuncture with standard blood collection technique. Samples were centrifuged at 3000 RPM for 10 minutes. Plasma was transferred to another labeled vial for immediate analysis or stored in deep freeze for analysis with in 24 hours. The plasma was used for the estimation of thiobarbituric acid reacting substances (TBARS) (Buege and Aust, 1978), Total antioxidant activity (TAA) (Benzie and strain, 1996) Vitamin C (Natelson, 1971), and α-tocopherol (Baker and Frank, 1978) by standard methods.

*Statistical analysis*

The results are reported as mean± SD. Statistical analysis was conducted with SPSS 16 version software using the 't' test with $p<0.05$ considered statistically significant.

## Results

Total cancer patients and NS age, plasma TBARS, TAA vitamin C and vitamin E are given in Table 1. The plasma TBARS was significantly raised (p=0.001) in cancer patients and TAA, vitamin E were significantly low (p=0.001) in total cancer patients when compared to NS.

In Figure 1a) there was no significant differences in the vitamin C of the smoker cancer patients when compared to smoker NS. The plasma TBARS was significantly raised and vitamin E was significantly low in smoker cancer patients when compared to smoker NS. Figure 1b) shows that plasma vitamin E was significantly low ($p< 0.05$) in non smoker cases when compared to non smoker NS.

In both smokers and non smoker cancer patients TAA was significantly low when compared to NS smokers and non smokers (Figure 2). But one interesting observation is that great reduction in TAA was observed in cancer patients who were non smokers (426±159). This level was even lower than the TAA of smokers (581±222) and the NS smokers and non smokers (732±187, 854±152).

## Discussion

Heavy smoking is widely prevalent in Nepal and lung cancer is one of the most common cancers in Nepal in both genders (Binu et al., 2007). As per WHO report it

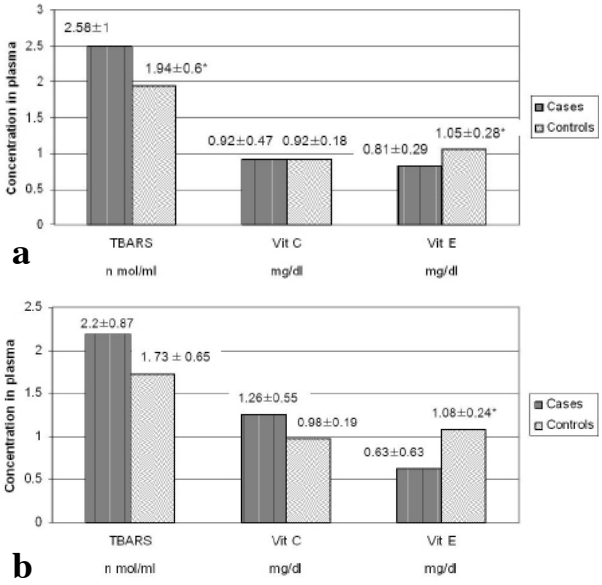


**Figure 1. TBARS, Vitamin C and Vitamin E Levels in Cases and Controls. a) Smokers; b) Non-Smokers** * p value < 0.05

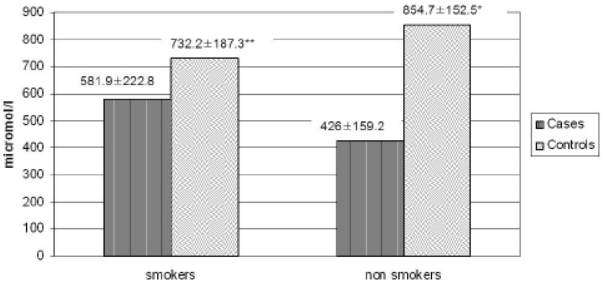


**Figure 2. TAA Levels in Smoking and Non Smoking Cases and Controls** *,** p value <0.001, <0.05

is 61.5% and 34.6% in males and females respectively (Mackay and Menash, 2004). A recent survey by the world society reforms and overall development services center Nepalgunj (Nepal) revealed that this country spends a staggering sum of NRs 24 billion per year on booze and fags, and that each family spends as NRs 20000/- on cigarettes. These oxidants react with various cellular components and lead to cancer development. There is clear evidence that cigarette smoking plays an important role in the genesis of lung, laryngeal, pharyngeal and oral cancer (Sasco et al., 2004; Gandini et al., 2008; Faux et al., 2009).

We found general increase in TBARS concentration in plasma of all the cancer patients. However the level of TBARS concentration was significantly increased in cancer patients with smoking habit when compared to controls with smoking habit. This is mainly due to ROS and reactive nitrogen species (RNS) produced by the smoke, ensure the mutations in DNA, which mainly affects the balance between proliferation and apoptosis.

**Table 1. Antioxidant Status and Oxidative Stress in Cases and Controls**

| | n | Age± SD (yrs) | TBARS n mol/ml | TAA μmol /l | Vit.C mg/dl | Vit. E mg/dl |
|---|---|---|---|---|---|---|
| Cases | 42 | 61.2±10.2 | 2.4±0.97* | 537.4±216.8* | 1.02±0.51 | 0.76±0.42* |
| Controls | 43 | 56.7±19.1 | 1.8±0.62 | 780.7±182.8 | 0.95±0.19 | 1.06±0.26 |

Cases Vs Controls* p value = <0.001

This uncontrolled proliferation of the cells leads to tumor development. The raised levels of TBARS suggest that the natural defense mechanisms are not adequate to inactivate ROS completely. Arivazhagan et al., (1997) had demonstrated that nicotine plays an important role in cancer development by inhibiting apoptosis. There are numerous reports in the literatures indicating raised oxidative stress in cancer (Skryzydlewska et al., 2005; Pasupathi et al., 2009; Burlakova et al., 2010). Our observations indicating that smoke induced oxidative stress plays an important role in cancer development.

Since ROS generation is a continuous phenomenon of aerobic life and could be toxic, man has evolutionarily developed an efficient network of enzymic and non-enzymic network of antioxidants as a guarding device. Thereby in this study we estimated non-enzymatic antioxidans vitamin C, vitamin E and total antioxidant activity (TAA). No statistically significant change was observed in the vitamin C level of total cancer patients with smoking and without smoking habit when compared to NS. Interestingly smoking did not influence vitamin C level. Risal et al., (2006), Adhikari et al., (2005) observed that there were no significant differences in vitamin C level in smokers and non smokers when compared with controls. The main reason seems to be the liberal consumption of green vegetables especially leafy ones throughout the year which are relatively inexpensive or often available free of cost and is fondly mixed with most vegetables and also with non vegetarian preparations. Nepali population also has a good habit of consuming inexpensive seasonal fruits such as oranges and apples.

Vitamin E is effective chain breaking lipid soluble antioxidant, thus helping to maintain membrane stability. It protects critical cellular structure against damage caused by ROS and reactive products of lipid peroxidation. Vitamin E levels were significantly decreased in total cancer patients, smokers and non smokers when compared to counterparts. More vitamin E is utilized to reduce oxidative stress. Abiaka et al., (2001) reported that $\alpha$-tocopherol concentration decreased significantly in total cancer, stomach, colon, rectal and breast cancer cases. Two cohort studies found significant inverse association between dietary intake of vitamin E and risk of lung cancer with the habit of cigarette smoking (Yong et al., 1997; Woodson et al., 1999).

TAA is the antioxidant strength contributed by nutrients and other antioxidants in the diet. TAA level was significantly decreased in total cancer, smoker and non smoker cancer patients when compared to counterpart controls. This is indicating that more consumption of dietary antioxidants other than vitamin C by tumors to scavenge free radicals. Sener et al. (2006) revealed that breast cancer patients had higher lipid peroxidation levels and lower blood total antioxidant capacity. In another study pleural effusion culture of lymphocytes from carcinoma patients presented lower levels of total antioxidant capacity and higher degree of DNA oxidative damage (Liu et al., 2003). Great reduction in TAA was observed in cancer patients who were non smokers. This level is even lower than the TAA of smokers and the normal subjects. This indicates that cancer development is closely associated with greater reduction of TAA in smokers and that may be due to increased oxidants introduced by smoking. Furthermore, the development of cancer in non smokers is associated with a greater reduction of TAA level over and above that of smokers. This may be due to massive reduction of endogenous antioxidant levels in non smoking cancer patients.

In conclusion, in view of the data indicate that smoking induces lipid peroxidation and weak antioxidant defense mechanism could a major risk factor in cancer development. Further research should attempt to establish the role of smoking in association with oxidative stress and antioxidants in Nepali population before come to a conclusion.

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