RESEARCH ARTICLE

Influence of Alcohol Consumption on Oxidative Stress and Antioxidant Status in Cancer Patients - Case-control Study from Western Nepal

T Nagamma[1]*, Rinchen Doma Bhutia[2], Daya Ram Pokharel[3], Saraswati Yadav[4], J Baxi[5]

Abstract

Aim: The present study assess the effect of consumption of alcohol on oxidative stress and antioxidant status in patients suffering from different types of cancer. Methods: This hospital based case control study conducted in the Western part of Nepal covered a total of 93 cancer patients with or without alcohol intake and smoking habits, along with 94 age, sex and habit-matched individuals serving as controls. Plasma thiobarbituric acid reacting substances (TBARS), total antioxidant activity (TAA), vitamin C, α-tocopherol and erythrocyte reduced glutathione (GSH) were estimated and compared. Results: The TBARS level was found to be significantly higher ($p \leq 0.001$) in all types of cancer patients when compared to controls, being aggravated in alcoholics with a smoking habit. No statistical significance ($p \geq 0.05$) was observed in the level of vitamin C and α-tocopherol. GSH and TAA level were significantly decreased ($p \leq 0.001$) in all the groups except those who consumed both branded as well as homemade alcohol and non-alcoholics without smoking habit. Conclusion: Alcohol, irrespective of its commercial brand, increases oxidative stress in all types of cancer patients. This is even higher when alcohol intake is combined with a smoking habit. Decreased TAA and GSH are major risk factors for cancer development.

Keywords: Alcohol - oxidative stress - antioxidants - cancer - Western Nepal

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Introduction

Alcoholism represents one of the most serious worldwide socioeconomic and health problems. Approximately 389,000 cases of cancer representing 3.6% of all cancers (5.2% in men and 1.7% in women) derive from alcohol consumption in the world (Rehm et al., 2004). Beside ethanol, alcoholic beverages contain some other alcohol such as methanol, isopropanol and ethyl glycol and also more than 4000 chemical substances which are linked to liver diseases and cancers (Kaplan and Pesce, 1996). Heavy smoking and alcohol drinking is widely prevalent in Nepal. A survey conducted by the World Society Reforms and Overall Development Service Centre in 2005 revealed that each family spends NRs 40000/- on liquor annually. Although exact incidence of different cancers in Nepal is not yet available, preliminary studies suggest that lung, oral cavity and stomach in males, and cervix, breast and lung in females are the most common type of cancers in Nepal (Binu et al., 2007; Pradhananga et al., 2009)

Alcohol promotes the generation of reactive oxygen species and interferes with the body's normal defense mechanism. 80-90% of alcohol breakdown in the liver results in the formation of acetaldehyde whose further metabolism in the cells leads to reactive oxygen species production (ROS) (Kim and Shin, 2002; Pronko et al., 2002). Acetaldehyde itself is a mutagenic and carcinogenic by product. It binds with DNA and interferes at DNA synthesis and repair mechanism, further it results in tumor development (IARC, 2010). Alcohol also stimulates the activity of enzymes called cytochrome P450 isoenzyme (CYP2E1). CYP2E1 activates numerous procarcinogens found in liquors and smoking significantly increases the risk of esophageal and laryngeal cancer (Samir, 2006). Further, alcohol can alter the levels of certain metal ions in the body that facilitates the increased production of ROS. The ROS so produced result in the formation of lipid peroxidation products such as malonialdehyde and 4-hydroxy 2-nonenal which either chemically modify or damage the cellular proteins and DNA and thereore play an important role in the development of alcohol related liver cancer (Worral and Thiele, 2001; Tuma, 2002).
Chronic alcohol consumption is a risk factor for certain types of cancer including esophagus, larynx, pharynx, liver, colorectum and breast (Zambon et al., 2000; Jedrychowski et al., 2001; Su and Arab, 2004; Mckillop and Schrum, 2005; Voigt, 2005; Garavello et al., 2006;

*[1]Department of biochemistry, Melaka Manipal Medical College, Manipal, [2]Department of Biochemistry, Sikkim Manipal Institute of Medical Sciences, Sikkim, [4]Department of Physiology, Narayana Medical College, Nellore, India, [3]Department of biochemistry, [5]Department of Surgery, Manipal College of Medical Sciences, Pokhara, Nepal *For correspondence: nagu7890@yahoo.co.in*

Key et al., 2006).

The aim of this study was to evaluate the oxidative stress and antioxidant status in cancer patients with reference to alcohol consumption from western region of Nepal.

Materials and Methods

The study was conducted on 93 cancer patients of various sites from the department of oncology suffering from oesophagus (n=17), larynx (n=8), pharynx (n=3), liver (n=18), colorectal (n=11) and breast cancer (n=36). This study included newly diagnosed cases as well as patients suffering from cancer with duration of one to two months. Detailed present and past history of name, age, sex, height, weight, BMI, cancer site, therapy given (chemotherapy/surgery or any other), duration of suffering, drinking habit, smoking habit and socio-economic status were taken for the study. A detailed history regarding the habit of alcoholism, local alcohol intake and smoking was taken. We have defined alcoholics as the ones who consumed alcohol daily and the ones who consumed >80ml alcohol/day. Prior verbal consent was taken from all the subjects participated in the study.

In this study cancer alcoholics were divided into two sub groups. Group I of cancer patients included who were consuming >3 pegs of alcohol (consuming branded alcohol) per day (1Peg=40 ml) with alcohol habit 18±3 years. Group II of cancer patients included those who were consuming >4 pegs of alcohol plus local alcohol (Raksi) per day with a habit of 20±2 years. Non alcoholics had never taken alcohol. Fifty six cancer patient smokers smoking 8 -10 cigarettes per day for 19±2 were included in this study. Ninety four age and sex matched individuals without any known diseases and not receiving any antioxidant supplementation served as controls. Alcoholic controls included those individuals consuming 2-3 pegs of alcohol per day and 1-3 pegs of alcohol plus local alcohol with a habit of 15±2 years. Non alcoholic controls had never taken alcohol. Seventy eight control smokers smoking 5-6 cigarettes per day for 20±3 were included in this study. This study was conducted in Manipal Teaching Hospital, Pokhara, Nepal. Cancer patients who were on radiotherapy and any other illness were excluded from the study.

Local alcohol

It is the finest homemade fermentative product of millets (kodo) or rice. Quantity wise local alcohol consumption is the highest when compared to branded alcohol as the cost is low and also widely brewed in the households. No local data is available on diseases related to alcohol consumption.

Sample collection and Laboratory analysis

Six ml of venous blood was collected from each subject by venipuncture with standard blood collection technique and transferred to EDTA containing vial. Sample was mixed properly with anticoagulant and then the blood sample was transferred to acid washed centrifuge tube leaving one ml blood in the vial for reduced glutathione (GSH) (Buetler and Kelly, 1963) estimation. Sample was centrifuged for 10 minutes at 3000 rpm at room temperature. The plasma was transferred to another labeled acid washed vial and analyzed immediately or stored in deep freeze for further analysis. The plasma total antioxidant activity was immediately analyzed after separating the sample. The plasma was used for the estimation of thiobarbituric acid reacting substances (TBARS) (Buege and Aust, 1978), total antioxidant activity (Benzie and Strain, 1996), vitamin C (Natelson, 1971) and α-tocopherol (Baker and Frank, 1968) by standard methods.

Statistical analysis

The results are reported as mean±SD. Computer software program SPSS version 14 was used for the statistical analysis of different parameters. Independent samples 't' test was calculated and $p \leq 0.05$ was considered statistically significant.

Results

Table 1 shows the antioxidant status and peroxidative stress in total cases and controls. The plasma TBARS was significantly raised ($p \leq 0.001$), TAA and GSH were significantly low ($p \leq 0.001$). No significant change was observed in vitamin C and α -tocopherol level in total cases when compared to controls.

In group I and group II cancer patients TBARS was significantly raised ($p \leq 0.001$), TAA and GSH level were significantly low in group I and group II ($p \leq 0.001$) and non-alcoholics ($p \leq 0.05$). No significant change was observed in vitamin C and α-tocopherol level in group I and group II patients and in non-alcoholics when compared with their counterpart controls (Table 2).

In group I and group II cancer patients with smoking habit TBARS was significantly raised but in group I it was aggravated and significantly low TAA and GSH level were observed when compared with their counterpart controls. α- tocopherol was significantly low in group II patients with smoking habit.

Table 2 shows the comparison between alcoholics and non-alcoholics without smoking habit. The TBARS level was significantly raised in group II patients without smoking habit. TAA was significantly decreased in group I patients without smoking habit. GSH was significantly low ($p \leq 0.001$) in group I, group II and non-alcoholics without

Table 1. Antioxidant Status and Peroxidative Stress in Total Cases and Controls

Parameters	Cases (n=93) Mean±SD	Controls (n=94) Mean±SD
Age (years)	55.1 ±12.2	49.6 ±19.5
BMI (kg/m^2)	23.2 ±4	22.2 ±2.87
TBARS (nmol/ml)	3.3 ±1.73*	1.7 ±0.69
TAA μmol /l	564.6 ±240*	802.0 ±172
Vitamin C (mg/dl)	1.0 ±0.6	0.9 ±0.23
α- Tocopherol (mg/dl)	0.9 ±0.45	0.97 ±0.35
GSH mg/dl of RBC	10.9 ±2.5*	28.9 ±6.03

*p value = ≤0.001 Cases Vs Control

Table 2. Antioxidant and TBARS Levels in Cases and Controls with Respect to their Alcohol and Smoking Habit

	No.	Age	TBARS nmol /ml	TAA μmol /l	Vitamin C mg/dl	α- Tocopherol mg/dl	GSH mg/dl of RBC
Antioxidant and TBARS levels in group I, group II non alcoholic cancer patients and controls							
Alcoholics (Group I)							
Cases	40	57.8±8.9	3.97±1.5*	529±188*	1.10±0.6	0.90±0.49	10.72±2.7*
Controls	48	48.9± 20.9	1.7±0.67	810±173	0.88±0.25	0.91±0.33	28.8±6.3
Alcoholics + Local alcohol (Group II)							
Cases	30	53.2±12.1	3.1±1.76*	561±301*	0.86±0.4	0.77±0.4	11.2±2.5*
Controls	18	55.2±17.8	1.43±0.5	811±146	0.93±0.23	0.98±0.39	29.7±5.8
Non alcoholics							
Cases	23	52.7± 16.3	2.42±1.5	630±228**	1.20±0.7	0.90±0.47	11.04±2.22*
Controls	28	47.4 ±18.1	1.8±0.75	780±189	0.98±0.18	1.06±0.34	28.7±5.9
Antioxidant and TBARS levels in group I and group II with smoking habit cancer patients and controls							
Group I with smoking habit							
Cases	28	58.7±9.6	4.06 ±1.6*	541±244*	1.08±0.8	0.90±0.7	10.6±2.71*
Controls	45	50.1± 20.9	1.7±0.68	805±175	0.87±0.25	0.89±0.33	28.5±6.5
Group II with smoking habit							
Cases	28	53.2±12.2	2.8±2.06*	627±220*a	1.17±0.65	0.78±0.4**	11.03±.2.84*
Controls	33	51.8±17.3	1.5±0.71	810±172	0.98±0.21	0.98±0.48	28.5±6.05
Comparison between group I, group II and non alcoholics without smoking habit and controls							
Group I without smoking habit							
Cases	16	54.3 ±86.0	3.8 ±1.3	461±422*	0.97±0.44	0.98±0.53	11.0± 2.4*
Controls	2	26.5±2.1	2.25±0.63	825±35	1.00±0.20	1.30±0.14	32.5± 0.7
Group II without smoking habit							
Cases	12	51.8±15.0	2.8±0.88*	618±417	0.79±0.31	0.90±0.36	11.6±2.3*
Controls	5	44.0±22.9	1.4±0.61	856 ±124	0.89±0.23	1.17± 0.44	30.0±5.7
Non alcoholics without smoking habit							
Cases	9	55.1±19.5	1.9±0.78	551±185	1.14±0.7	0.99 ±0.55	11.0±1*
Controls	9	47.5±19.5	2.12±0.58	719±200	0.93±0.1	1.13±0.31	31.1±4

*p value = ≤0.001, **p value = ≤0.05 compared to controls

smoking habit when compared with their counterpart controls.

Discussion

Alcohol consumption is the world's third largest risk factor for diseases. Approximately 2.5 million deaths result each year from the chronic and excessive use of alcohol. Worldwide per capita consumption of alcoholic beverages equal to 1.76 liters of homemade or illegally produced (local alcohol). According to WHO recent report total adult per capita consumption among all drinkers in Nepal was 10.5 (WHO, 2011). The higher the consumption of alcohol, the greater the risk for colorectal, larynx, liver, esophagus, oral cavity and pharynx, female breast cancer (Baan et al., 2007). Even consumption of two drinks per day causes an increased risk for breast cancer (Hamajima et al., 2002).

We found that there was a significant increase in oxidative stress (OS) in total cancer patients (3.31±4), group I (3.97±1.5), group II (3.1±1.76) and group II patients without smoking habit (2.8±0.88) when compared with controls. The overall increase in OS was due to reactive oxygen species and reactive nitrogen species produced by alcohol consumption directly related with development of pathogenesis like cancer or the alcoholic liver disease (Sergent et al., 2001; Wu, 2003). The raised TBARS indicates the weak antioxidant defense system against raised OS. There were numerous reports indicating raised OS in cancer patients due to alcohol consumption (Terry et al., 2001; Poschl and Seitz, 2004; Rainis et al., 2007; Ha et al., 2010; Manjunath et al., 2010). Not only the commercial alcohol but also the combination of alcohol and commercial alcohol consumption plays an important role in increased OS and further in cancer development. Surprisingly OS was more pronounced (4.06±1.6) in group I patients with smoking habit. This aggravating effect of alcohol may be due to alcohol itself partly converted to free radicals because it has a solvent effect for the compounds present in smoke and therefore enhances their absorption or it modifies the chemical species present in tobacco which then accelerates the peroxidative process or due to combined effect. Dey and Caderbaum (2006) reported that alcohol increase the ROS pool in smokers. There are reports that support the view that alcohol and smoking together increases risk of cancer (Vioque et al., 2008; Ming et al., 2011).

Vitamin C and α-tocopherol are said to be infrangible friends in antioxidant arena. They can mutually exchange the unpaired electron plucked from free radicals. Both can operate together in extracellular and intracellular fluids and as symbiotic neighbours at cellular or membrane interphase, but more often electron flow from α-tocopherol towards ascorbic acid because α-tocopherol is intercalated in lipid layer of biomembranes to serve as security guard against lipid peroxidation. No statistical significance was observed in vitamin C and α-tocopherol level of group I and group II patients when compared with controls. In our previous study we did not find any singnificant difference in vitamin C level in cancer patients when compared to

controls (Nagamma et al., 2011). Several previous studies have observed that there are no significant differences in vitamin C and α-tocopherol level in alcoholics, alcoholics with and without smoking habit in Nepali population (Adhikari et al., 2005; Risal et al., 2006; Jha et al., 2007). The normal vitamin C and α-tocopherol level can be due to liberal consumption of inexpensive, seasonally available leafy vegetables and fruits along with non-vegetarian diet. These plant products are endowed with numerous phytochemical beset with antioxidant properties and in fact many of them act as broad spectrum antioxidants. The normal levels of vitamin C and α-tocopherol suggest that in total antioxidant cascade, there are antioxidants other than vitamin C and α-tocopherol which are consumed on priority basis to meet the exigencies of smoke. Significantly low level α-tocopherol was observed in group II patients with smoking habit when compared with counterpart contols. No data is available on effect of local alcohol. So presently we cannot answer for the rise of α-tocopherol in group II smokers but undoubtedly these interesting queries have paved the way for future researches in this direction.

Total antioxidant activity (TAA) is a better method for non-enzymatic antioxidant activity. The normal range of TAA by this method is 600- 1600 μmol/l. It does not determine the antioxidant activity of plasma proteins and –SH compounds including glutathione. TAA level was significantly decreased in group I and group II patients, group I and group II patients with smoking habit and also in group I patients without smoking habit when compared with controls. Su et al. (2001) found that poor and well differentiated gastric adenocarcinomas patients had decreased level of total antioxidant capacity. Similarly decrease in blood antioxidant capacity was also observed in breast cancer patients (Sener et al., 2006). Ching et al. (2002) in a case and control study revealed that women with high TAA had 53% lesser risk to develop breast cancer.

Reduced GSH efficiently scavenges free radicals and other reactive oxygen species like hydrogen peroxide, hydroxyl, lipid peroxyl radicals and peroxy nitrite. In our study we have observed that significantly decreased GSH level in all the cases when compared to counterpart controls. Several studies have been reported that chronic alcohol consumption has been associated with increased lipid peroxidation and decrease in GSH (Lu, 2000a; Lieber., 2002; Das and Vasudevan, 2005) In another study it was observed that patients with fibroadenoma and adenocarcinoma of the breast had increased oxidative stress and decreased concentration of GSH (Kumaraguruparan et al., 2002).

In conclusion, our findings suggest that Nepali population is not exception to the rule-of-thumb that chronic alcoholism leads to increased oxidative stress and low total antioxidant level in the body and that is alcoholism when combined with smoking further aggravate this condition. Moreover, our findings also suggest that chronic effect of alcoholism is same irrespective of the commercial brand or method of brewing of alcohol. As our study is a hospital based and confined only to western part of Nepal, a more detailed study encompassing the representative population from the various parts of the country needs to be carried out to generalize the overall effect of chronic alcoholism in Nepalese population. We believe that our study results serve as baseline data to plan such studies in future.

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