RESEARCH ARTICLE

Non-enzymatic Antioxidant Status and Biochemical Parameters in the Consumers of Pan Masala Containing Tobacco

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Abstract

Background: Tobacco consumption is one of the leading causes of oral submucous fibrosis, oral cancer and even premature death. The present study was designed to compare the biochemical parameters and non-enzymatic antioxidant status and the lipid peroxidation products in pan masala tobacco users as compared with age-matched non-user controls. Methods: Pan masala and tobacco users of age 33.2±9.94 years and age-matched controls (31.2±4.73 years) were enrolled for the study. Plasma levels of vitamin E, vitamin C, albumin, bilirubin, uric acid, glucose, urea, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT) were measured by standard methods. Serum malondialdehyde (MDA) levels were estimated as a measure of lipid peroxidation. Results: In the pan masala tobacco users, as compared to the controls, the level of vitamin C (68.5±5.9 vs 97.9±9.03 μmol/L, p≤0.05) vitamin E (18.4±5.3 vs 97.9±9.03 μmol/L, p≤0.001), albumin (37.5±7.01 vs 44.3±9.99 g/L, p≤0.001), and malondialdehyde (10.8±1.29 vs 1.72±1.15 nmol/ml, p≤0.001) were found to be significantly altered. Malondialdehyde was significantly correlated with vitamin E (r=1.00, p<0.001) and vitamin C (r =1.00, p<0.001) in pan masala tobacco users. Serum levels of AST (31.0±16.77 IU) and ALT (36.7±31.3 IU) in the pan masala tobacco users were significantly raised as compared to the controls (AST, 25.2±9.51 IU, p=0.038; ALT, 26.2±17.9 IU, p=0.038). Conclusion: These findings suggest that pan masala tobacco users are in a state of oxidative stress promoting cellular damage. Non-enzymatic antioxidants are depleted in pan masala tobacco users with subsequent alteration in the biochemical parameters. Supplementation of antioxidants may prevent oxidative damage in pan masala tobacco users.

Keywords: Pan masala tobacco - antioxidants - Nepal

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Introduction

Tobacco consumption is one of the leading causes of oral submucous fibrosis, oral cancer and even premature death (Gupta and Ray, 2004; Rai et al, 2011). It is a socially acceptable habit in the two third population of developing countries including Nepal (Gupta and Ray, 2004; Sreeramareddy et al., 2008; Turk et al., 2012). Tobacco and related products collectively termed as pan masala tobacco (PMT) is openly distributed in retail shops, and is readily available for consumption in Nepal. In Nepal, an estimated 4.1 deaths per day are due to tobacco related diseases, which accounts for 1500 deaths annually (Pande 2001; Sah, 2007). Among the three ecological regions of Nepal, overall tobacco usage prevalence is 68.2% in the high hills, 42.4% in the terai, and 40.9% in the low hills (Pande, 2001). A recent study conducted in college students of Western Nepal, showed 21.32% prevalence of tobacco users. Among the students, 42.9% used pan masala, 20.7% used gutka and 8.1% used tobacco only respectively (Subba et al., 2011). Tobacco is used in conjunction with betel quid, and areca nut as the chief psychoactive substance, if direct tobacco is not added (Pednekar, 2003; Gupta and Ray, 2004). Chemical constituents of betel quid include: a) alkaloids, b) polyphenols, c) tannins, d) trace elements including sodium, magnesium, chlorine calcium, vanadium, manganese, copper and bromine and, e) reactive oxygen species (Sharan et al., 2012). Pan masala popularly known as 'gutka' is a commercial preparation containing areca nut, slaked lime, catechu and condiments with or without tobacco, which is dehydrated so that the final product is imperishable (Pednekar, 2003). Use of 'gutka' and PMT is a common modality of tobacco usage and especially popular among the youths (Sushma and Sharang, 2005; Sreeramareddy et al., 2008).

The recent investigations into cell culture studies have demonstrated that long-term usage of PMT and tobacco related products are the potential generators of free radicals. The highly reactive radicals and reactive oxygen

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species (ROS) can act as initiators of carcinogenesis, cause DNA damage, activate pro-carcinogens and alter the cellular antioxidant defence system (Avti et al., 2006). Changing the balance towards an increase in the pro-oxidants over the capacity of the antioxidants is defined as oxidative stress, which might lead to oxidative damage. Non-enzymatic anti-oxidants albumin, uric acid, bilirubin, vitamin C and vitamin E act in concert to reduce the oxidative damage by scavenging free radicals and by detoxifying the oxidants. Malondialdehyde (MDA) the end product of lipid peroxidation by ROS is used as the biomarker of oxidative stress (Kohen and Nyska, 2002). The present study was designed to compare the biochemical parameters and non-enzymatic antioxidant status and the lipid peroxidation products of the PMT consumers as compared with age-matched controls.

Materials and Methods

The present study was conducted in Department of Biochemistry, B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal from June 2009 to August 2010. Snowball sampling technique was used for the enrollment of the subjects. Informed verbal and written consent was obtained from the study subjects. Total of 50 PMT consumers who had no severe disease and 53 age matched PMT non-user controls were enrolled for the study. Blood samples were collected in EDTA tubes and plain vials, for obtaining plasma and serum samples respectively. Blood samples were centrifuged for 10 min at 3000 RPM and stored at -20 ºC, the tests were performed within 24 hours of blood collection. Plasma vitamin C (Sullivan, 1995), vitamin E (Bieri, 1964) and serum malondialdehyde (MDA) (Yagi, 1987) were estimated by spectrophotometric methods. Total bilirubin (Malloy and Evelyn, 1937) and albumin (Doumas et al., 1971) were estimated by Malloy-Evelyn and BCG methods. Glucose(Koch and Nipper, 1977), urea(Lespinas, et al., 1989), creatinine(Crocker et al., 1988), aspartate amino transferase (AST) and alanine amino transferase (ALT) (Reitman and Frankel, 1957) were estimated using standard kits. All biochemical tests were performed using Vitalab Selectra E Autoanalyzer. Ethical Clearance was taken from Institutional Ethical Review Board of BPKIHS.

Statistical Analysis

Data were represented as Mean±SD. Student 't' test was applied to compare non-parametric variables at 5% level of significance. Pearson's Correlation coefficient was determined at 5% level of significance. The data were analysed by SPSS version 11.5 (SPSS Inc. USA). P value less than 0.05 was considered as statistically significant at 95% confidence intervals.

Results

Among PMT users 94% were male and 6% were female similarly among PMT non-user controls 94.34% were male and 5.66% were female respectively. PMT users had mean age 33.24±9.94 years and controls had mean age 31.17±4.730 years. Among the PMT consumers 34% consumed 3 to 5 packets (12-20 gm) of PMT per day whereas 32% consumed 5 to 10 packets (20-40 gm), 14% consumed 10-15 packets (40 -60 gm), 10% consumed 15-20 packets (60-80 gm) and remaining 10% consumed more than 20 packets (>60 gm) per day respectively.

Table 1 shows the comparison of biochemical parameters among PMT users and PMT non user controls. In the PMT users, there was significant rise in serum content of AST (30.95±16.77 IU) and ALT (36.74±31.27 IU) as compared to controls AST (25.19±9.51 IU, p=0.038), ALT (26.24±17.93 IU, p=0.038) respectively.

Table 2 shows the various non-enzymatic antioxidants in PMT users and PMT non-user controls. PMT users had significantly lower serum albumin levels (37.54±7.01 g/L) as compared to controls (44.28±9.99 g/L, p<0.001). Total bilirubin was found to be significantly raised in PMT users (17.42±11.24 μmol/L) as compared to controls (13.23±6.95 μmol/L, p=0.024). The results showed significant increase in the levels of circulating MDA, a marker of lipid peroxidation in PMT users (10.78±1.29 nmol/ml) than that in controls (1.72±1.15 nmol/ml, p<0.001). In the PMT users, vitamins C (68.52±5.90 μmol/L) was significantly lower as compared to controls (97.87±9.03 μmol/L, p=0.008). Similarly, vitamin E was found to be lower in PMT users (18.37±5.39 μmol/L) as compared to controls (40.02±3.7 μmol/L, p<0.001) respectively. Table 3 shows the correlations of MDA with various vitamin and non-enzymatic antioxidants in PMT users, controls and total subjects. Vitamin C (r =1.00, p<0.001) and vitamin E (r =1.00, p<0.001) levels in PMT

Table 1. Comparison of Different Biochemical Parameters in PMT Users and Controls

Parameter	PMT users (mean ±SD)	Controls (mean ±SD)	p value
Glucose (mmol/L)	93.54±18.80	96.40±21.06	0.471
Urea (mmol/L)	18.73±3.77	20.08±5.59	0.154
Creatinine (μmol/L)	0.827±0.25	0.835±0.118	0.84
AST (IU/L)	30.95±16.77	25.19±9.51	0.038
ALT (IU/L)	36.74±31.27	26.24±17.93	0.038

Table 2. Comparison of Non-enzymatic and Vitamin Antioxidants in PMT users and PMT Non-users

Parameters	PMT users	Controls	p value
Albumin (g/L)	37.54±7.01	44.28±9.99	< 0.001
Total Bilirubin (μmol/L)	17.42±11.24	13.23±6.95	0.024
Uric acid (μmol/L)	244.47±13.57	249.52±9.16	0.758
Vitamin C (μmol/L)	68.52±5.90	97.87±9.03	0.008
Vitamin E (μmol/L)	18.37±5.39	40.02±3.7	< 0.001
MDA (nmol/ml)	10.78±1.29	1.72±1.15	< 0.001

Table 3. Comparison of Non-enzymatic and Vitamin Antioxidants in PMT users and PMT Non-users

Parameters	Correlation coefficients		
	PMT users	Controls	Total
Albumin	0.073	-0.409*	-0.379**
Total Bilirubin	-0.169	0.033	0.142
Uric acid	0.85	0.011	-0.005
Vitamin C	1.00**	-0.93	-0.101
Vitamin E	1.00**	-1.58	-0.21*

*p value<0.05, **p value<0.001

users were significantly correlated with MDA. Albumin (r=-0.379, p <0.001) and vitamin E (r =-0.21. p=0.03) were significantly correlated with MDA in total subjects (Table 3).

Discussion

The present study showed significant decrease in levels of Vitamin C and E in PMT users as compared to PMT non-user controls. Vitamin C is regarded as the first line natural antioxidant defence in plasma and a powerful inhibitor of lipid peroxidation. It also regenerates the major antioxidant tocopherol. Vitamin E acts as a chain breaking antioxidant, which can directly scavenge a variety of oxy-radicals, including peroxy (ROO^-), hydroxyl (OH^-) and superoxide (O_2) radicals respectively. Thus, our finding indicates a possible role for ascorbic acid and tocopherol as antioxidants in PMT users (Burton, 1989).

Non-enzymatic Antioxidants such as glutathione, vitamin E (α–tocopherol), vitamin A (retinol), vitamin C (ascorbic acid) carotenoids, thioredoxin, lipoic acid, and ubiquinone and the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase act in concert to protect against the free radical-induced damage, mutagenesis and carcinogenesis (Devasagayam et al., 2004; Halliwell, 2007).

Our study showed that bilirubin was significantly increased, while albumin was significantly decreased. The compensating mechanism of free radical scavenging and its association with the increased formation of bilirubin may be due to free radicals induction of gene for biliverdin reductase. Secondly, in PMT users there occurs intoxication of liver, which confers to the increased levels of bilirubin. Uric acid the final product of purine degradation, acts as an antioxidant by virtue of its ability to tightly bound iron and copper (Davies et al., 1987). Although, no significant differences in uric acid levels was found between the two groups, the temporal order of antioxidant consumption in human blood plasma exposed to a constant flux of aqueous peroxy radicals is vitamin C > bilirubin > uric acid > vitamin E (Cochrane, 1991).

PMT is a mixture of areca nut, tobacco, lime, catechu and spices and other condiments, and its chemical analysis have shown the presence of poly-aromatic hydrocarbons, nitrosamines and toxic elements lead, cadmium and nickel. The intoxication by these compounds induce the microsomal cytochrome P450, a source of ROS. Superoxide anion and hydrogen peroxide are the ROS, which emerge in particular, following the breakdown and uncoupling uncoupling of the cytochrome P450 catalytic cycle. These ROS cause lipid peroxidation and oxidative damage (Kohen and Nyska, 2002; Subapriya et al., 2003; Sushma and Sharang, 2005).

The antioxidant system comprises of different types of functional components classified as: i) preventive antioxidants: which reduce the rate of chain initiation and ii) chain breaking antioxidants, which interfere with the chain propagation. The antioxidants belonging to first category are enzymes such as SOD, catalase, glutathione and those belonging to second line of defence include vitamin C, uric acid, albumin, bilirubin, vitamin E and carotenoids respectively (Burton, 1989; Carr and Frei, 1999). Our study, showed significant increase in level of MDA in PMT users as compared to controls. MDA a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids, is used as an indicator of tissue damage by a series of chain reactions (Kohen and Nyska, 2002).

In the present study, we observed significant increased level of AST and ALT in PMT users as compared to PMT non-user controls. In contrast, glucose, urea and creatinine were not significantly increased. Serum transaminases, ALT and AST are sensitive indicator of liver cell injury. Elevated level of ALT and AST in PMT users might be related to damage and destruction of the liver tissue as PMT contains ingredients of hepatotoxic agent which induces microsomal enzyme of liver cells (Burtis et al., 2006).

Subba et al, 2011 showed significant association between tobacco chewing and gender, income group, age, smoking, alcohol use, having ≥3 chewer friends and family's chewing habits respectively (Subba, 2011). In a study conducted in rats by Pramod et al (2005) it was found that long-term administration of aqueous extract of smokeless tobacco impairs enzymatic antioxidant defence system and reduced glutathione levels (Avti et al., 2006). Subapriya et al. (2003) reported enhanced lipid peroxidation accompanied by depleted antioxidants in patients with oral pre-cancer and cancer (Subapriya, et al., 2003).

Pre-cancerous conditions such as oral leukoplakia (OL), oral erythroplakia or submucous fibrosis are found in vast majority of betel quid users and higher risks are reported in pan masala users (Trivedy et al., 2002; Sharan et al., 2012). Several previous studies have shown that individual constituents of betel quid and areca nut, or in addition to slaked lime, are both carcinogenic and detrimental to health. (Rai et al., 2011)

Antioxidants have protective roles with the progression of various devastating diseases like cancer, cardiovascular diseases and diabetes. Balance has to be maintained between antioxidant protectors and components that promote oxidation in the body, which subsequently relates to sound health and disease progression (Davies et al., 1987; Subapriya et al., 2003). The enzymatic antioxidants, including catalase, superoxide dismutase and glutathione peroxidase and their roles can be studied in a larger community based interventional studies using vitamin supplementation in PMT users as a further scope of this study. Mass media campaigns for tobacco control should be addressed to the vulnerable groups, and a focus should be given to gender inequalities, for awareness regarding cessation of smoking for women and to control the consumption of PMT.(Turk et al., 2012)

In conclusions: Our study concludes that PMT users are at high risk of free radical damage and accumulation of lipid peroxidation products (MDA). Non-enzymatic antioxidants (Vitamin C, Vitamin E, Albumin, Billirubin) are depleted with consequent increase in the levels of AST and ALT in PMT users. Depletion of antioxidants are risk-factors for cancer, coronary heart disease and other severe chronic diseases. Awareness programs regarding

the detrimental effects of chewing pan and tobacco related products should be conducted in populations. Antioxidants levels and lipid peroxidation products should be frequently monitored in people consuming PMT and antioxidants should be supplemented regularly through diet which may certainly aid to prevent the oxidative damage caused by PMT usage.

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