Asian Pac. J. Health Sci., 2017; 4(3):14-19 e-ISSN: 2349-0659, p-ISSN: 2350-0964

Document heading **doi: 10.21276/apjhs.2017.4.3.4** **Research Article**

Serum ascorbic acid and alpha-tocopherol levels in bidi smokers and tobacco chewers

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Received: 20-06-2017 / Revised: 30-06-2017 / Accepted: 27-07-2017

ABSTRACT

Aim: To assess serum ascorbic acid and alpha-tocopherol levels in bidi smokers, tobacco chewers and tobacco non-users. **Materials and Methods**: Forty smokers [25 subjects smoking ≤24 bidi /day (moderate) and 15 subjects smoking > 24 bidi/day (heavy) with a smoking habit of > 10 years, 20 subjects tobacco chewers ≥10 g tobacco/day) chewing for > 10 years] were included in the study.All selected subjects were normal with no symptoms of any disease. They were classified on the basis of age, BMI and with and without alcohol consuming habit. Serum ascorbic acid was measured by dinitrophenyl hydrazine reagent and alpha-tocopherol by ferric chloride oxidation.
Result: Serum ascorbic acid levels in moderate smokers, heavy smokers, tobacco chewers and control,were 0.31 -+0.13, 0.26+0-.16, 0.40+0.2-6 and 0.50+-0.09.In the respective group's alpha-tocopherol levels were 0.674±0.46, 0.94±0.43, 0.95±0.61 and 1.39±0.73.**Conclusion:** Both bidi smoking and tobacco chewing decreased ascorbic acid and alpha-tocopherol levels. No discernible trends were found with BMI and alcohol.

Keywords: Ascorbic acid, tobacco, BMI.

Introduction

The use of tobacco is common all over the world. Initially it came as a health stimulant but in last 5-6 decades there is gradual increase in demonstrating its dangerous destructive and deadly effects. The tobacco leaf contains about 3700 toxic chemicals which may go up as high as 4700 during its processing as smoked or smokeless tobacco. Out of these two, smoking is more harmful affecting almost all organ with preference to aero digestive space. Smokeless tobacco also affects all organs but with less intensity compared to smoking and that its effects are mainly confined to oral cavity,

The present evidence suggests that major infliction caused by tobacco is through free radical species and that this adverse effect is potentiated with weaker antioxidant defense in the body.

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Several workers have reported that tobacco users tend to consume more a diet relatively poor in antioxidant; that the free radical species of tobacco consume more antioxidants thereby decreasing the level of body's antioxidant defense; that dietary antioxidant provide a good protective antioxidant support; and among them alpha- tocopherol and ascorbic acid serve as first line of defense.

The above observations are reported among cigarette smokers. To best of our knowledge similar studies on bidi smokers, whose use is 7 time more prevalent than cigarette in India, are not recorded in literature. The present study reports the ascorbic acid and alpha-tocopherol status among moderate and heavy bidi smokers. This study is further extended on tobacco chewers who consumed more than 10 g tobacco per day as data on them with, regard to ascorbic acid and alpha-tocopherol is still meagre and none so far in the state of Rajasthan.

Materials and methods

Eighty male healthy volunteers in the age group thirty to sixty years, using tobacco for> 10 years were included in the study with their prior consent. Of these 20 were tobacco non-users, 20 tobacco chewers and25 persons smoking ≤24 bidi/day (one bundle/packet}/day and 15 persons smoking> 24 bidi/day. All of them smoked two types of bidis, depending on the availability. One was 7.6 cm long with an average tobacco content of 247.2±8.68 mg (average of 5 bidis) and the other 6.4 cm long with average tobacco content of 189.4±12.36 mg (average of 5 bidis). However, the major consumption was of smaller size BIDI. A detailed history was recorded on separate performa regarding general information i.e., age, height, weight, BMI, religion, occupation, dietary habits, alcoholism, socio-economic status, history suggestive of any illness, hospitalization, drug intake, etc. A detailed history regarding the habit of tobacco use i.e., smoking or smokeless tobacco use was taken.

The subjects were classified on the basis of age, BMI and regular (daily) intake of alcohol. BMI was calculated by following formula:

BMI = Weight in kg/Height in Metre^2

Fasting blood samples of all subjects were collected and serum was analyzed for ascorbic acid calorimetrically by dinitrophenyl hydrazine method and alpha-tocopherol by oxidation with ferric chloride (Baker and Frank, 1968) [1].

Results

We observed following salient features in our study. The ascorbic acid level in bidi smokers was significantly lower than tobacco chewers and non-users while no significant decrease in tobacco chewers and non-users and also ascorbic acid level was much lower in subjects who smoke> 24 bidis day. Significantly low level were found in young tobacco chewers. No relation with BMI and alcohol was observed.

Ascorbic acid level decreases in following order:

Tobacco chewer >: ≥ 24 bidi/day >>24 bidi /day. It was lowest in persons consuming > 24 bidi /day.

Alpha-tocopherol level were also significantly lower in tobacco chewer than non- users. Along with BMI lean tobacco chewers had significantly lower alpha-tocopherol level.

Alpha-tocopherol level was lowest in < 24 bidi/ day order in tobacco chewer>>24 bidi /Zday ><24 bidi/day. These findings were strengthen by ANOVA test and Bon Ferroni procedure.

Table 1: Serum levels of ascorbic acid and alpha –tocopherol

Subjects	N	Ascorbic acid (mg/dl)	Alpha-tocopherol(mg/dl)
Tobacco non-users	20	0.50±0.09	1.39±0.73
Bidi smokers (≤24 bidi/day)	25	0.31±0.16	*0.66±0.46*
Bidi smokers (>24 bidi/day)	15	0.26±0.16	*0.94±0.43*
Tobacco chewers	20	*0.40±0.26*	0.95±0.61

Table 2: Distribution according to age

Subjects	Ascorbic acid (mg/ dl)		Alpha-tocopherol (mg/ dl)	
Age	< 45 years	> 45 years	< 45 years	> 45 years
Tobacco chewers	0.25±0.19(n= 11)	0.58±0.21(n=9)	0.97±0.75(n=ll)	0.92±0.44(n=9)
Bidi smokers ≤24 + > 24 bidi/day	0.27±0.12(n=23)	0.32±0.17(n=17)	0.87±0.44(n=23)	0.63±0.47(n=17)

Table 3: Distribution according to BMI

Subjects	Ascorbic acid (mg/ dl)		Alpha-tocopherol (mg/ dl)	
BMI	≥18.5	<18.5	≥18.5	<18.5
Bidi smokers ≤24+ > 24 bidi/day	0.27±0.12 (n=26)	0.33±0.17 (n= 14)	0.86±0.52 (n=26)	0.59±0.27(n= 14)
Tobacco chewers	0.5l±0.28 (n= 12)	0.33±0.22 (n=8)	1.30±0.76 (n= 12)	0.71+0.37 (n=8)

Table 4 :According to alcohol consuming habit

Subjects	Ascorbic acid (mg/ dl)		Alpha-tocopherol (mg/ dl)	
Habit of Alcohol	alcohol consuming	Non-alcohol consuming	alcohol consuming	Non-alcohol consuming
Bidi smokers ≤24+ > 24 bidi/day	0.26±0.15 (n=10)	0.27±0.09 (n= 30)	0.73±0.58 (n=10)	1.39±0.73 (n= 30)
Tobacco chewers	0.29±0.24 (n= 6)	0.27±0.09 (n=14)	0.75±0.45 (n= 6)	1.39±0.73 (n=14)

Table 5: Statistical analysis by ANOVA test (analysis of variance) for ascorbic acid (mg/dl)

Source of variation	SS	Df	MS	F ratio	F crit;
Between groups	0.6184	2	0.3092	10.7361	3.07
Within Groups	2.2205	77	0.0288		
Total	2.8389	79	pvalue<0.05 S		

Table 6: Statistical analysis by ANOVA test (analysis of variance) for alpha-tocopherol (mg/ dl)

Source of variation	SS	Df	MS	F ratio	F crit;
Between groups	5.3298	2	0.3298	8.0803	3.07
Within Groups	25.39	77	2.6649		
Total	30.72	79	pvalue<0.05 S		

Table 6: BON FERRONI procedure for means of three groups of ascorbic acid

Comparison group	't' statistic'	Critical value of 't' statistic at 0.984 Level of significance (one tailed test) = 2.374
Bidi smoker and tobacco chewer	2.3275	Non-Significant
Bidi smoker and total non-user	4.5689	Significant
Tobacco chewer and non-user	1.9402	Non-Significant

Table 7: BON FERRONI procedure for means of three groups of alpha-tocopherol

Comparison group	't' statistic'	Critical value of 't' statistic at 0.984 Level of significance (one tailed test) = 2.374
Bidi smoker and tobacco chewer	1.1545	Non-significant
Bidi smoker and total non-user	4.0171	Significant
Tobacco chewer and non-user	2.4778	Significant

Discussion

Our work is based on the premise; that tobacco is harmful to health, leading to injury on a scale varying from morbidity to mortality; that tobacco smoke is more harmful than the smokeless tobacco; that the inhaled smoke originating from the combustion of tobacco contains a wide variety of chemical substances, other than nicotine, which exert serious noxious effects specially in the causation of the disease; that many of these species are free radicals and that their range can be diminished or expurgated to a limited degree by antioxidants; but can never be completely contained in chronic tobacco users. In general, it is abundantly proved that antioxidant functions are associated with diminished lipid peroxidation, better DNA and protein protection or inhibition of LDL oxidation and malignant, transformation in vitro. Epidemiological studies support the hypothesis that antioxidants lower the incidence of many types of cancers, CVD cataracts and chronic inflammatory diseases (Gutteridge and Halliwell, 1996)[2]. Antioxidants also provide a better protection against respiratory disorders in, smokers (Wei et al., 2001 and Kim and Lee, 2001) [3, 4]. Clinical' and animal studies further strengthen these

presumptions [Caderas and Parks, (2002) [5]. Indeed there is gathering evidence that even under physiologic conditions body tissues, cells and extra and intra-cellular fluids arc being constantly hammered by pro-oxidant challenges and the body has evolved matching system of antioxidant defense that uses a combination of various strategies which consist of enzymatic and non-enzymatic -curriculum. An impressive share of non-enzymatic defense is accomplished by essential dietary micro-nutrients and undoubtedly ascorbic acid and alpha-tocopherol playa seminal role in this consortium.

In this series the mean level of ascorbic acid in serum of matched non-smokers was 0.50±0.09 mg/ dl. The normal ascorbic level in plasma should be in the range of 0.5-1.5 mg/ dl and for its optimal antioxidant' activity the level should be; 1.1 mg/ dl. Two conclusions are very much obvious from our observations. First, the matched controls, who belonged to lower socio-economic group had the level on the lowest scale of normal range and that the individual value revealed that 45.0% of control had levels below normal (<0.5 mg/dl). Notably none of the subjects had the value ≤1.1 mg/dl. This is expected also for several reasons, first, they belonged to deprived class or verging it and therefore could not afford a balanced food, second, they neither had financial capacity to choose, nor knowledge to exercise choice among available foods and lastly they usually spend money for tobacco at the expense of their diet. The mean level of serum ascorbic acid in 40 smokers was 0.29±0.14 mg/dl and was not only significantly lower than controls but almost depleted. Interestingly, when bidi smokers were divided into two groups viz., < 24 bidi smokers and >24 bidi smokers, the latter group had, significantly lower vitamin C status (0.26±0.16 mg/dl) than the former (0.31±0.13 mg/ dl). Thus our findings conclusively suggest that bidi smoking enfeebles ascorbic acid status and this adverse effect is more pronounced in heavy smokers. Statistical findings affirm this deduction. We further examined the influence of alcohol and relationship of EMI with plasma ascorbic acid levels among smokers. Neither of these parameters showed any association. Similar type of evaluation in bidi smokers from other populations is not available in literature but there are some reports on cigarette smokers. Lykkesfeldt et al. (2000) concluded from their study that smoking depletes ascorbic acid [6]. Dietrich et al. (2002) investigated the supplementation of various antioxidants for two months viz., ascorbic acid alone and ascorbic acid + alpha-tocopherol + alpha-lipoic acid on oxidative stress in smokers [7]. The daily intake of 500 mg ascorbic acid lowered the oxidative stress and intriguingly the combination with ascorbic acid was less effective, despite the fact that alpha-lipoic acid is considered to be a broad spectrum antioxidant. This emphasizes the recent assertion that antioxidants are never universal, are organ specific and under some given conditions may even exert oxidant activity. Traber et al (2000) reviewed the role of antioxidants in tobacco related diseases and stated that both ascorbic acid and alpha-tocopherol are significantly decreased in heavy smokers due to lower intake or stimulated utilization or both [8]. Mezetti et al. (1995) reported fluorescent products of lipid was significantly raised in smokers and was associated with lower level of both these vitamins [9]. Mandez et al. (2002) exclusively studied ascorbic acid status in smokers and non-smokers and came to conclusion that smokers are at greater risk of chronic diseases due to low intake of ascorbic acid[10]. The observations of Preston et al (2003) are still more important in this regard who reported that ascorbic acid status is compromised in smokers and that environmental tobacco smoke reduces ascorbic acid status even when exposure to it is minimal[11]. Likewise the adverse effects of smoking on ascorbic acid status is reported in chronic obstructive pulmonary diseases

(Calikoglu et al, 2002)[12], passive smoking (Ayaori et a., 2000)[13], pregnancy (Ortega et al 1998)[14], cancer, cardiovascular diseases and in many other pathologies. Our subjects were not having any apparent symptoms of the disease out were frail and our observation of lower level of ascorbic acid along with clinical observations suggest that these persons were relatively in more vulnerable zone than non-smokers.

Ascorbic acid and alpha-tocopherol are said to infrangible friends in antioxidants arena. They can mutually exchange the unpaired electron plucked from free radicals. Both can operate together in extracellular and intracellular fluids and as symbiotic neighbors at cellular or membrane inter-phase, but more often electron flows from alpha-tocopherol towards ascorbic acid because alpha-tocopherol is intercalated in lipid layer of bio membranes to serve as security guard against lipid peroxidation and preferentially accepts the unpaired electron before they could hurt the lipid species in membranes and then passes on its congener ascorbic acid and others in cytosolic phase for final disposal. The antioxidant effects of alpha- tocopherol are well recorded in literature and there are several reports about its supplemental value in cigarette smokers but the data are not available on bidi smokers. In our study the alpha-tocopherol level among controls (non-smokers) was 1.39±0.73 mg/dl. The smokers had significantly lower level (0.77±0.47 mg/dl). In the two

groups of smokers viz., moderate and severe smokers the level was 0.66±0.46 mg/dl (<24 bidi/day) and 0.94±0.43 mg/dl (>24 bidi/ day). The difference between these two groups was statistically non-significant. No relationship was observed with BMI and alcohol.

We further extended our study to examine the status of these two antioxidant vitamins in tobacco chewers, which is the commonest mode of. Usage of smokeless tobacco in 'this region. All the subjects took the tobacco with lime, lacing the paste in between lower set of teeth and lips and then sucking it intermittently. Normally the quid was replaced at the interval of 2-3 hours,all of them had the habit of keeping the quid during sleep also. Various harmful effects of use of smokeless tobacco are reported in literature from India and elsewhere (WHO Atlas, 2000) [15].

Some of the toxic species are asserted to be free radicals in smokeless tobacco also. Further, these are suggestedto be generated in oral cavity and other tissues after absorption and antioxidant supplementation could be useful (Traber et al., 2000) [8]. As regard to antioxidant status, we did not observe any significant difference in ascorbic acid level between tobacco chewers, (0.40±0.26 mg/dl] and tobacco non- chewers (0.50±0.09 mg /dl] but the plasma alpha-tocopherol level was significantly low (tobacco chewers 0.95±0.61 mg/dl and tobacco non-users 1.39±0:73 mg/dl), though the decrease in any case cannot be categorized as depleted status. In this regard some studies need specific mention. For example Bagchi et al. (2001) [16] observed that the smokeless tobacco induces oxidative stress, thereby modulating Bel-2 and P-53 genes in human oral cavity which my lead to oral cancer and that ascorbic acid, alpha-tocopherol, combination of alpha-tocopherol +C and grape seed extract containing antioxidant proanthocynaidin and others offered 11%, 26%, 28% and 50% protection against oxidative damage. Driskell et al. (1996) noted that tobacco smokers, chewers and non-users having similar intake of vitamin showed the following gradient in plasma levels non-users > chewers > smokers [17]. There are several other reports supporting the aforesaid claims but' few contrary reports arc also there (Stegmayer et al., 1993). May be final verdict is obtained with longitudinal studies in due course of time [18].

Conclusion

Bidi smoking is more harmful than tobacco chewing; that smoking significantly reduces the ascorbic acid status and tends to decrease alpha-tocopherol status; that heavy smokers (> 24 bidi] show same trend compared to moderate smoker (< 24 bidi/day); that smoking and BMI or alcohol consumption relationship is frail one; that tobacco chewing has lesser influence on alpha-tocopherol and ascorbic acid status than smoking; that with age chewing inversely affects only ascorbic acid status and that both these vitamins are anointed antioxidants for protecting against smoking hazards. The present study reinforces that tobacco in any form is injurious to health and refocuses this issue, which is raking the world community with justified distressing health alarm.

Acknowledgement

We would also like to give my thanks to all colleagues in department supports. Furthermore we wish to give my special thanks to all those patients enrolled in study and their relatives for their outstanding support and cooperation to conduct this study.

Ethical approval

This study was an observational study. The investigations required were a part of routine management of illness. No change was made in usual management of subjects under study, informed consent was taken from the patients who were enrolled for study. In case of refusal of consent, subjects continue to receive the standard treatment as per protocol.

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Source of Support: Nil
Conflict of Interest: Nil