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EFFECTS OF ACTIVE AND PASSIVE SMOKING ON ANTIOXIDANT ENZYMES AND ANTIOXIDANT MICRONUTRIENTS

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ABSTRACT

In the present study, we analyzed the relationship between cigarette smoke exposure and several markers of oxidative status, plasma thiobarbituric acid reactive substances, erythrocyte glutathione peroxidase, superoxide dismutase and catalase in a group of students. Of the 105 men enrolled into the study, 35 had never smoked and not exposed to cigarette smoke at all. Thirty five had smoked at least 15 cigarettes per day for at least five years (active smokers) and 35 had been exposed to cigarette smoke at indoor environment at least 2 cigarette/day on ≥5d/wk for > 6 months (passive smokers). The urine cotinine level was used as a smoking marker. Erythrocyte SOD activity and plasma TBARs were significantly higher in active and passive smokers than in non-smokers ($p < 0.05$). However, erythrocyte GSH-Px and CAT were significanly lower in active smokers than in non smokers ($p < 0.05$). Serum vitamin C and E levels were significantly lower in active and passive smokers than in nonsmokers ($p < 0.05$). For active and passive smokers, there were significant possitive correlations between urine cotinine levels and plasma TBARs levels ($r = 0.60$, $p < 0.01$, $r = 0.43$, $p < 0.05$) and a negative correlation between urine cotinine levels and plasma vitamin C ($r = -0.48$, $p < 0.05$, $r = -0.59$, $p < 0.01$). In conclusion, during both passive and active smoking, oxidative stress was clearly exacerbated and the dynamic balance between oxidation and antioxidation was seriously disrupted, which was closely related to many disorders or diseases in active and passive smokers.

Introduction

According[1] to meta-analysis of epidemiological studies, there is an elevated risk of coronary heart disease and lung cancer for both active and passive smokers (1, 2, 3, 4, 5). The extent of the environmental tobacco smoke (ETS) related risk in passive smokers is also estimated to be as high as experienced by active smokers. A number of mechanisms may be involved in the atherogenesis induced by passive smoking, such as dysfunction of endothelium (6, 7), altered lipoprotein profiles (8, 9), increase in oxidizability of low-density lipoprotein (LDL), and decrease in plasma ascorbic acid (AA) levels caused by ETS exposure (10). Increased lipid peroxidation products have been observed in atherosclerotic plaques (11), and it has been suggested that smoking could induce atherosclerosis in part through the formation of oxidatively modified lipids and/or the generation of oxidative stress in the vascular wall. Tobacco smoke contains numerous com-

[1] **Abbreviations:** Thiobarbituricacid reactive substance (TBARs), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH-Px), Vitamin C , Vitamin E

pounds, many of which are oxidants and prooxidants, capable of producing free radical and enhancing the oxidative stress (12, 13, 14). Studies have shown that tobacco smoking can also alter oxidative enzyme activities (15, 16, 17). It has not only been reported that active smoking was associated with increased lipid peroxides in plasma, but also that ETS exposure affected plasma lipid peroxidation (10). There is also an increasing evidence that the intake of dietary antioxidant vitamins, such as vitamin C, vitamin E, and β-carotene, is associated with a decreased risk of atherosclerosis (8, 10, 11, 14). Smokers have lower blood levels of these antioxidant vitamins (10).

There are a few researches putting forth relation between urine cotinine and plasma oxidant for consideration in active and passive smokers. To this extend level of lipid peroxidation and alteration in antioxidative enzyme activities in passive smokers and comparison with active smokers have been evaluated in this study. The relationship between the antioxidant enzyme activities, vitamin-E and C levels and urine cotinine concentrations as an objective marker of smoke inhalation was investigated in active and passive smokers.

Materials and Methods

Subjects

Blood and urine samples were obtained from students .105 men were enrolled into the study. Informed consent was taken from the participants of the study. Subjects consisted of 105 healthy males who read, understood, and answered a questionnaire and signed the informed consent forms. Smoking habits and ETS exposure were evaluated by using a smoking-history questionnaire that allowed an assessment of the hours of ETS exposure in the worksite and the home. According to the results of the questionnaire, the subjects were divided into three groups, 35 of them (19-31 years) had never smoked and were not exposed to cigarette smoke at all, 35 (20-29 years) had smoked at least 15 cigarettes per day for at least five years who were current smoker and had not quit smoking (active smokers), and 35 (18-30 years) were exposed to cigarette smoke at indoor environment of ≥ 2 cigarette/day on ≥ 5day/week for > 6 months (passive smokers). Subjects were questionned for vitamins and alcohol intake which might change oxidant state potently. Subjects who were taking alcohol, ascorbic acid, α tocopherol or multivitamin supplements during the last month before participating in the study were excluded. Another exclusion criteria for participation in the study were based on the reported consumption of ≥ 4 serving of fruit and vegatables/per day. Subjects were fasted after midnight before blood collection in the next morning.

Sample preparation and analysis

10 ml venous blood was collected in heparinized syringes and centrifuged at 3000 rpm for 10 min to separate the plasma from the erythrocytes. Plasma was harvested, and red cells were washed in an excess of physiologic saline solution. Plasma was used for measurement of TBARs. The cells were then haemolysed in distilled water (1:5, v/v) and by the freezing-thawing procedure. The hemolysate was centrifuged (22000 x g 60 min) and the supernatant was used to determine superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GSH-Px) activity and haemoglobin concentration (cyanomethemoglobin).

Erythrocyte SOD was measured by modified Winterbain and Hawkins' method based upon reduction of nitroblue tetrasolium (NBT) (18). One unit of SOD activity is defined as the amount of the protein that inhibits the rate of NBT reduction by 50 %. Enzyme activity was expressed as units per g. of Hb (U/g Hb).

CAT activity in haemolysate was assayed by a method described by Aebi (19). One unit CAT activity was defined as the amount of enzyme which liberates half

TABLE 1

Demographic data between the groups

	Nonsmokers (n=35)	Passive Smokers (n=35)	Smokers (n=35)
Age, years	25±6	24±6	26±4
Weight, kg	58.2±7.2	56.8±6.1	57.7±6.8
Height, m	1.71±0.05	1.72±0.09	1.70±0.08
Marital status, married (n)	5	6	4
Urine cotinine (μmol/l)	75.3±4.6	160.3±8.74	467.8±69.3

peroxide oxygen from an H_2O_2 solution in 100s at 25 °C. Enzyme activity was expressed as units per g. of Hb (U/g Hb).

GSH-Px activity in erythrocytes was measured by the method of Paglia and Valentine with t-butyl hydroperoxide as substrate and expressed in units/g of haemoglobin (U/g Hb). One unit of GSH-Px activity represents 1 μmol- t-butyl hydroperoxide / min under the defined assay conditions (20).

TBARS concentrations were quantified spectrophotometrically. To prevent the interference of haemoglobin and its derivatives (maximum absorbance at 540 nm) during the measurement of TBARS (maximum absorbance at 532 nm), a method described by Pyles et al.was used (21). The TBARS concentration was expressed as nmol/ml using a molar extinction coefficient of $1.56x10^5$ M^{-1} cm^{-1} at 535 nm . All samples were analysed in duplicate, the mean value of which was taken as the final result.

Total ascorbic acid was measured spectrophotometrically as chromogen by using 2,4 dinitrophenylhydrazine (22). Plasma α-tocopherol level was determined by spectrophotometric method (23).

Urine samples were collected in glass containers for the determination of cotinine. All samples were stored at –20°C until analysis. A commercially available kit was used for measurement of urinary cotinine (24).

Statistical Analysis

All statistical analysis was performed with the computer program namely statistical package for the social science (SPSS) for Windows, version 8.0. The following statistical procedures were used; the comparison of the variables according to groups was performed by using the one way ANOVA test. Tukey-HSD (Honest Significantly Different) and Tamhane tests were used for multiple comparisons of the variables. To determine the significance of interactions between various variables in each group, linear correlation and regression analysis was performed.

Results and Discussion

Table 1 shows the demographic data between groups. Coefficient of variations for these measurements are shown in **Table 2**. **Table 3** shows the biochemical values of antioxidant enzymes, MDA, vitamin C, vitamin E, and urine cotinine in active and passive smokers and in control subjects. Active smokers had urinary cotinine levels higher than those affected by the environmental smoke. Erythrocyte SOD activity and plasma TBARs were significantly higher in active and passive smokers than in non-smokers ($p < 0.05$). Erythrocyte GSH-Px and CAT were significantly lower in active and passive smokers than in non smokers ($p < 0.05$). Serum vitamin-C and E levels were significantly lower in active and passive smokers than in non smokers ($p < 0.05$) (Table 3).

For both active and passive smokers, there were significant positive correlations between urine cotinine and plasma TBARs ($r= 0.60$, $p < 0.01$, $r=0.43$, $p < 0.05$ respectively) and a negative correlation between

TABLE 2

Coefficient of Varaitions of the Measurements

	Within day(n:10)		*Day-to-day(n:10)*	
	Mean	*%CV*	*Mean*	*%CV*
Erythrocyte SOD (U/g Hb)	1418	2.1	1492	2.3
Erythrocyte GSH-Px (U/g Hb)	61.3	1.9	65.8	2.8
Erythrocyte CAT(U/g Hb)	625.8	2.4	663.2	2.9
Plasma TBARs (nmol/ml)	4.8	0.8	5.8	2.4
Plasma Vitamin-C (mg/dl)	0.681	1.8	0.692	1.8
Plasma Vitamin-E (mg/dl)	0.38	1.6	0.41	3.1
Urine cotinine (μmol/l)	108.3	2.4	128.4	3.2

TABLE 3

Comparison of plasma, erythrocyte and urine parameters between active smokers, passive smokers and nonsmokers

	Nonsmokers (n=35)	*Passive Smokers (n=35)*	*Smokers (n=35)*
Erythrocyte SOD (U/g Hb)	1438±73.6[a]	1608±68.1[a]	1698±92.6[a]
Erythrocyte GSH-Px (U/g Hb)	60±7.21[a,b]	55.8±6.18[b]	51.67±6.85[a]
Erythrocyte CAT(U/g Hb)	621±50.09[a,b]	543.3±63.2[b]	537.1±27.55[a]
Plasma TBARs (nmol/ml)	2.092±0.184[a,b]	8.975±1.49[b]	9.883±2.55[a]
Plasma Vitamin-C (mg/dl)	0.728+0.043[a]	0.632+0.064[a]	0.601+0.053[a]
Plasma Vitamin-E (mg/dl)	0.434±0.04[a]	0.282±0.05[a]	0.236±0.02[a]
Urine cotinine (μmol/l)	75.3±4.6[a]	160.3±8.74[a]	467.8±69.3[a]

[a, b,] : The differences between mean values with same superscripts at each row is significant (at least $p < 0.05$).

TABLE 4

Correlations between urine cotinine and other parameters (vitamin C, E, antioxidant ezymes activity) in active and passive smokers

	Active Smokers		*Passive Smokers*	
With urine cotinine	r	P	r	P
Erythrocyte SOD	0.22	$p > 0.05$	0.17	$p > 0.05$
Erythrocyte CAT	0.15	$p > 0.05$	0.19	$p > 0.05$
Plasma Vitamin-C	-0.48	$P < 0.05$	-0.59	$P < 0.01$
Plasma Vitamin-E	-0.18	$p > 0.05$	-0.25	$p > 0.05$
Plasma TBARs	0.60	$P < 0.01$	0.43	$P < 0.05$

urine cotinine and plasma vitamin-C levels ($r = -0.48$, $p < 0.05$, $r = -0.59$, $p < 0.01$ respectively) (**Table 4**).

Cigarette smoking has been implicated in the pathogenesis of many diseases including coronary hearth disease, pulmonary disease, and neoplastic disorders (25). Besides cigarette smokers, passive smokers are also exposed to get harmful effects of tobacco (26, 27). The adverse action of the cigarette smoke is due to the presence of a large variety of compounds that could initiate or amplify oxidative damage (25, 26).

Various organs may control or prevent the damaging effects of the oxidant species by enzymatic and non-enzymatic antioxi-

dant defence systems. Specific enzymes like superoxide dismutase (SOD), catalase and GSH-Px have a crucial role in these antioxidant defences. Tobacco smoke can alter antioxidative enzyme activities. Certain vitamins like α-tocopherol (28) and ascorbic acid (29) are also suggested to have strong free radical scavenging properties.

In the present study it has been shown that there was a positive correlation between TBARs and urine cotinine as an objective marker of passive smoking. Increased SOD activity has occurred as a result of increased lipid peroxidation. However, erythrocyte CAT activity was lower in active and passive smokers than in controls ($p < 0.05$). These controversial results may be due to a compensation mechanism of the body. Decreased CAT activity may either be due to structural features of CAT or environmental factors. We did not detect a correlation between plasma urine cotinine and SOD or CAT activity. Husain et. all have also observed decrease in hepatic CAT activity after chronic administration of nicotine which could be related to oxidative inactivation of enzyme protein (30). They also concluded that a compensatory increase in antioxidant enzymes would be desirable. As H_2O_2 is liposoluble and neutral, it can simply pass through the cell membrane. It will lead to $OH^{\cdot}$ radical production, which is the main step in lipid peroxidation. The triggering mechanism of that event is the Fenton reaction, which leads to production of $OH^{\cdot}$ and OH^{-}. The next step is the peroxidation of the polyunsaturated fatty acids in the membranes or lipoproteins (31). CAT enzyme indirectly inhibits $H_2O_2 \rightarrow OH^{-} + OH^{\cdot}$ formation. Because enzymatic reactions in the body are faster than spontaneous reactions, H_2O_2 is catalysed into H_2O by CAT.

Erythrocyte GSH-Px activity was lower in passive smokers than in control ($p < 0.01$). CAT and GSH-Px are able to destroy H_2O_2, but GSH-Px has a much higher affinity for H_2O_2 than CAT, suggesting that H_2O_2 is principally degraded by GSH-Px in normal conditions. As GSH-Px detoxifies H_2O_2 with higher deficiency than CAT, it is not surprising to observe a good complementary effect with SOD, better than the combination of CAT and SOD. A combination of CAT and SOD leads to either positive or negative effects according to SOD concentration. Therefore SOD activity increases with the oxidative stress. Interestingly, in some cases, cells having higher SOD activity than normal also induce their GSH-Px activity and finally a decrease of CAT activity would be observed by a compensatory mechanism. In our study the decreased levels of CAT and GSH-Px activity in active and passive smoking groups may possibly be due to this mechanism. Conflicting results have been reported concerning the effects of smoking on these enzymes. Increased CAT levels have been reported, as well as the absence of its activity in smokers.

In vitro, prolonged exposure of plasma to gas-phase cigarette smoke causes depletion of antioxidants, including vitamins C and E (13, 17). In our study, plasma vitamin-C in passive smokers was found to be as low as that of active smokers and there was a significant decrease when compared with the control group ($p < 0.05$). There was a significant negative correlation between plasma vitamin-C and urine cotinine levels in both active and passive smokers. Vitamin C may protect against cigarette smoke damage by interfering with aqueous phase–Reactive oxygen species (ROS) originating from cigarette smoke rather than by inhibiting subsequent lipid peroxidation induced by ROS.

Smoking was more weakly associated with circulating concentrations of vitamin E (32). Supplemental vitamin E in smokers may raise plasma vitamin E, but this supplemental vitamin E may be removed faster from the plasma if oxidative stress is higher

in smokers. Yamaguchi et have suggested that the preventive effects of vitamin E can be effective against oxidative stress in smoking individuals (9). In our study, it was observed that plasma α-tocopherol levels of passive smokers was as low as that of active smokers and in both situations the values were significantly lower than in the control group ($p < 0.05$). Deprivation symptoms of this vitamin appear very late because of its storage in fatty tissues and easy supply in diet. There was a correlation between urine cotinine discharge and low plasma vitamin-C levels, but no such a relationship was shown for plasma vitamin-E levels. Thus, it can be suggested that vitamin-C is much more effective than vitamin-E in preventing events with oxidative stress.

The connection between smoking and dietary intake is extremely complex. The results indicate that any public health preventive action toward smokers should aim not only at suppressing tobacco use, but also at promoting better nutritional habits (33, 34, 35, 36). Although there is not enough evidence that indicates increased intake of antioxidant nutrients is beneficial, the observation that smokers have lower circulating levels of certain nutrients raises concern of such a need (35). Passive smokers may have a necessity of taking supplementary vitamin-C as much as active smokers (10).

As a result, it can be suggested that exposure to passive cigarette smoke especially in indoor environment may cause as similar oxidative stress as in active smoking. Any imbalance between the toxic radicals and antioxidant enzymes may cause passive smokers to have tendency to pathologic formations as much as active smokers due to structural and functional damages caused by vitamin deficiency.

REFERENCES

1. **Kawachi I., Colditz G.A., Speizer F.E., Manson J.E., Stampfer M.J., Willett W.C., Hennekens C.H.** (1997) Circulation, **95,** 2374-2379.

2. **Steenland K., Thun M., Lally C., Heath C.** (1996) Circulation, **94,** 622-628.

3. **Carmona R.H.** (2004) Health & Medical Guide new surgeon general's report expands list of diseases caused by smoking, 19-20.

4. **Eyre H., Kahn R.** (2004) Circulation, **109,** 3244-3255.

5. **Jemal A., Tiwari R.C., Murray T. et al.** (2004) Cancer. J. Clin., **54,** 8–29.

6. **Raij L., DeMaster E.G., Jaimes E.A.** (2001) J. Hypertens, **19,** 891-7.

7. **Heitzer T., Just H., Munzel T.** (1996) Circulation, **93,** 1488-1492.

8. **Valkonen M., Kuusi T.** (1998) Circulation, **97,** 2012–2016.

9. **Yamaguchi Y., Matsuno S., Kagota S., Haginaka J., Kunitomo M.** (2001) Atherosclerosis, **156,** 109-17.

10. **Tribble D.L., Giuliano L.J., Fortmann S.P.** (1993) Am. J. Clin. Nutr., **58,** 886–890.

11. **Mezzetti A., Lapenna D., Pierdemenico S.D.** (1995) Atherosclerosis, **112,** 91-99.

12. **Reilly M., Delany N., Lawson J.A.** (1996) Circulation, **94,** 19-25.

13. **Benowitz N.L., Kuyt F., Jacob F.** (1983) Clin. Pharmacol. Ther., **34,** 604-611.

14. **van Antverpen V.L., Theron A.J., Richards G.A., Steenkamp K.J., van der Merwe C.A. et al.** (1995) Free Radic Biol. Medicine, **31,** 935-941.

15. **Al-Senaidy A.M., Al-Zourang C.J.** (1997) Nutr. Health, **12,** 55-65.

16. **Zhou J.F., Yan X.F., Guo F.Z., Sun N.Y., Qian Z.J., Ding D.Y.** (2000) Biomed. Environ. Sci., **13,** 44-55.

17. **Codandabany U.** (2000) Cell. Biochem. Funct. **18,** 99-102.

18. **Sun Y., Oberley L.W., Li Y.** (1988) Clin. Chem., **34,** 497-500.

19. **Catalas A.H.** (1982) In: Methods in Enzymatic Analysis, (H.U. Bergmeger, Ed.), Verlag Chemie Weinheim, 3, 273-282.

20. **Paglia D.E., Valentine W.N.** (1967) J. Lab. Clin. Med., **70,** 158-69.

21. **Pyles L.A., Stejskal E.J., Einzig S.** (1993) Proc. Soc. Exp. Biol. Med., **202,** 407–419.

22. **Tietz N.W.** (1986) Textbook of Clinical Chemistry, W.B. Saunders company, 960-962.

23. **Martinek R.G.** (1964) Clin. Chem., **10,** 1078-86.

24. **Barlow R.D., Stone R.B., Wald N.J. Puhakainen E.V.J.** (1987) Clinica Chimica Acta, **165,** 45-52.

25. **Block G., Dietrich M., Norkus E.P., Morrow J.D., Hudes M., Caan B., Packer L.** (2002) Am. J.

Epidemiol., **156,** 274-285.
26. **Yildiz L., Kayaoglu N., Aksoy H.** (2002) Clin. Chem. Lab. Med., **40,** 612-5.
27. **Gurkan F., Kiral A., Dagli E., Karakoc F.** (2000) Eur. J. Epidemiol., **16,** 465-468.
28. **Brown K.M., Morrice P.C.** (1996) Clin. Scien., **91,** 107-111.
29. **Nam C.M., Oh K.W., Lee K.H., Jee S.H., Cho S.Y., Shim W.H., Suh I.** (2003) J. Am. Coll. Nutr., **22,** 372-8.
30. **Husain K., Scott B.R., Reddy S.K., Somani S.M.** (2001) Alcohol, **25,** 89-97.
31. **Yokus B., Cakir D.U., Akdag M.Z., Sert C., Mete N.** (2005) Free Radic. Res., **39,** 317-23.
32. **Anthony J., Alberg A.J.** (2002) Toxicology, **180,** 121-137.
33. **Vander V.A., Reznick A.Z., Crass C.E.** (2000) Clin. Chest. Med., 21**,** 173–187.
34. **Farque M.O., Khan M.R.** (1995) Br. J. Nutr., **73,** 625-692.
35. **Bolton S.C., Casey C.E., Smith W.S.C., Tunstol P.H.** (1991) Br. J. Nutr., **65,** 337-346.
36. **Kim S.H., Kim S.H., Shin H.S., Keen C.L.** (2003) Nutrition, **19,** 240-243.