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□Original Article□

Serum Antioxidant Vitamins and Erythrocyte Lipid Peroxide Levels in Female Adolescent Smokers*

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ABSTRACT

The purpose of this study was to investigate the association between adolescent smoking and antioxidant vitamins. Subjects were 87 non-smokers and 90 smokers, who were female high school students. Smokers were divided into two groups by smoking status, 35 light smokers(packyear<1) and 53 heavy smokers(packyear≥1). Dietary intakes were examined through questionnaires and nutrient intakes of vitamin C and A were analyzed by smoking status using Computer Aided Nutritional analysis program for professional (CAN-PRO). Serum vitamin C level was measured by 2,4-dinitrophenylhydrazine method and serum levels of vitamin A and E were measured by HPLC. Erythrocyte lipid peroxide level was measured by thiobarbituric acid reactive substance(TBARS) method. All data were statistically analyzed by SAS PC package program. The mean vitamin C intakes of non-smokers, light smokers and heavy smokers were 58.2mg/day, 50.1mg/day and 58.1mg/day, respectively. The mean vitamin A intakes of non-smokers, light and heavy smokers were 281.1μgR.E./day, 278.7μgR.E./day and 289.6μgR.E./day, respectively. There was no significant difference in dietary intakes of antioxidant vitamins by smoking status. However, the serum vitamin C level, 11.40mg/l in heavy smokers was 12% lower than that of 12.70mg/l in non-smokers. The serum vitamin A level was not significantly different among the groups. Vitamin E level, 8.79mg/l in heavy smokers was 8% lower than that of 9.53mg/l in non-smokers. There was no significant correlation between the dietary intakes and serum levels of vitamin A and C. The erythrocyte TBARS level, 1.90nmol/ml in light smokers was significantly lower($p<0.05$) than 2.71nmol/ml in heavy smokers or non-smokers. The correlation data showed that the erythrocyte TBARS level had a significant positive correlation with packyear. Overall results might suggest that cigarette smokers with a longer smoking history need more dietary intakes of vitamin C and E than do non-smokers to reach the same serum level. (*J Community Nutrition* 1(1) : 16~24, 1999)

KEY WORDS : female adolescent smokers · antioxidant vitamins · TBARS · nutritional status.

Introduction

Cigarette smoking is a well-known risk factor for both cardiovascular disease and lung cancer(Meng et al. 1991). Cigarette smoke contains a large number of oxidants, leading to oxidative damage of tissues. Cigarette smoke also contains large amounts of free radicals that could directly initiate and propagate the process of lipid peroxidation. It has been proposed that smoking may favor LDL oxidation in vivo(Harats et al. 1989). Thus, this phenomenon might contribute to the causative link between smoking and atherogenesis(Marangon et al. 1998).

Evidence that smokers are subject to oxidative stress includes the findings that smokers have lower concentrations of antioxidants than do non-smokers and the risk of coronary heart disease(CHD) in smokers correlates inversely with their intake of antioxidants (Thomson et al. 1992).

Cross et al.(1998) showed a depressed antioxidant nutrient status of the smokers which may be due to both their aberrant dietary habit and the increased demand placed on the antioxidants by cigarette smoke itself. The increased free-radical load incurred by smoking and lower intake of antioxidant nutrients may shift the normal free-radical antioxidant balance in the body with initiation of a deterioration process.

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Such an imbalance between prooxidants and antioxidants, linked to decreased smoke-related antioxidant capacity and increased free radical generation. This imbalance may be important in the etiology of cancer and cardiovascular diseases(Marangon et al. 1998). Because oxidative modification dominates current ideology concerning the pathogenesis of atherosclerosis and cancer, many studies have focused on oxidative stress as a probable clinically relevant factor in cigarette smoker-related atherogenesis and cancer(Cross et al. 1998).

Vitamin A, C and E are the most abundant and effective antioxidants in human serum and is believed to be of major importance in the protection against diseases and degenerative processes caused by oxidative stress(Frei 1989 ; Niki 1991). Smoking causes changes in the index of antioxidant vitamins status(Duthie et al. 1991).

Particularly, cigarette smoking has been known to be associated with a depletion of the ascorbic acid pool. Although smokers have a lower dietary intake of ascorbic acid than non-smokers, the ascorbic acid depletion by cigarette smoking appears to occur predominantly via a mechanism independent of dietary intake. Decreased plasma vitamin C concentration reported in smokers(Kallner et al. 1981) has been regarded as a consequence of greater vitamin C turnover in response to a sustained oxidant load rather than a decreased dietary intake(Duthie et al. 1991).

According to the report of "National smoking status" of Korea Institute for Health and Social Affairs(KIHASA) in 1995, increased smoking population in women and adolescents has become a social problem. Recently, the smoking rate of adolescent females have more rapidly increased than that of adolescent males. Taking into accounts of the role of the mother in the future, it will be a serious public health problem for adolescent females to smoke because smoking by a pregnant woman affects the health of herself, her fetus and her family. In addition, since it is reported that the period of smoking greatly affects incidence of lung cancer, therefore, smoking such as adolescence will also be a significant problem to the health of later life(Park 1989).

The purpose of this study was to investigate the effect of adolescent female smoking on nutritional status of antioxidant vitamins and lipid peroxide levels as an index of oxidative stress in high school students.

Subjects and Methods

1. Subjects

Data were collected from 90 female adolescent smokers and 87 female adolescent non-smokers attending high school in Seoul. Height and weight were measured, dietary data were obtained through questionnaires and serum samples were obtained. Smokers were categorized as light smokers(n=35) whose packyear is below 1 year and heavy smokers(n=53) whose packyear is equal to or above 1 year. Students who took medication and multivitamin supplementation, suffered from any chronic disease, or had problems with blood collection which resulted in insufficient samples for carrying out the analyses were excluded from the study. The subjects' characteristics were presented in the previous publication(Kim et al. 1999).

2. Dietary intakes

Dietary intakes were assessed by 3-day food record method. Nutrient intakes were analyzed by Computer Aided Nutritional analysis program for professionals(CAN-PRO ; 1997).

3. Sample preparation

Venous blood was collected into heparin treated syringes and heparin non-treated syringes after a twelve-hour fast. Tubes were kept on ice until they arrived at the laboratory. Blood samples were allowed to clot at room temperature for approximately 1 hour. Ten ml of blood samples was centrifuged at 3000rpm for 20 minutes at 4℃ to separate serum. Ten ml of blood samples was centrifuged at 1500rpm for 15min at 4℃ to separate red blood cells. Erythrocytes were washed 3 times with an ice cold, phosphate buffered saline solution and refrigerated. The serum samples were frozen under nitrogen and stored at −80℃ until analyzed.

4. Analysis of serum vitamin C

The serum vitamin C was assayed immediately after serum separation. The serum was deproteinized with 0.75M meta-phosphoric acid and measured by 2,4-dinitrophenylhydrazine method using a UV-Spectrophotometer(KONTRON, Uvikon 930) at 520nm(Pesce & Kaplan 1987).

5. Analysis of serum vitamin A and E

Serum levels of vitamin A and E were assessed by

measuring retinol and α-tocopherol, respectively. Serum vitamin A and E were extracted with ethyl alcohol and hexane. Retinol and α-tocopherol were separated by HPLC on Nova-Pak C_{18} (3.9×150mm) column using methanol-water(95 : 5, v/v) as the mobile phase. Elution was detected spectrophotometically at 292nm(Bieri et al. 1979).

6. Erythrocyte lipidperoxide level and serum lipids

Serum triglyceride and cholesterol were measured by previously published procedures(Kim et al. 1999). The level of erythrocyte lipid peroxide was assayed by TBARS method(Stocks & Dormandy 1971). Standard material was 1,1,3,3,-tetraethoxypropane(TEP). Each sample was mixed with 30% trichloroacetic acid and centrifuged at 2000rpm for 15 minutes. After centrifugation, the samples were mixed with 1% thiobarbituric acid in 0.05M NaOH and then boiled for 15 minutes. The absorbance was measured at 535nm with a spectrophotometer. The concentration was calculated by using the extinction coefficient of 1.56×10^5M.

7. Statistical analysis

All data were expressed as mean±SE. Means among groups were compared by Duncan's multiple range test. Correlations between smoking status and serum parameters were analyzed by Pearson's correlation test. Statistical significant differences were accepted at $p<0.05$. SAS-PC software was used for all the analyses.

Results and Discussion

1. Smoking status and packyear in smokers

Average packyear as concerned to 1 pack/day was calculated by the amount of smoking(cigarettes/day) multiplied by duration of smoking(year) in 90 smokers. Since the subjects were female adolescents with short packyear, smokers were categorized as light smokers whose packyear is equal to or below 1 year and heavy smokers whose packyear is equal to or above 1 year. Percentages of light smokers and heavy smokers were 41%(n=35) and 59%(n=53), respectively. Among heavy smokers, percentage of smokers whose packyear are above 2 years was 19.3%.

In light smokers, the average number of cigarettes smoked was 2.9(cigarettes/day), the average duration of smoking was 2.1 years and average packyear was 0.32 years. In heavy smokers, the number of cigarettes smoked was 10.6(cigarettes/day), the average duration of smoking was 3.6 years and average packyear was 1.9 years (Table 1).

Table 1. Cigarette smoking status of female adolescent smokers

	Light smokers[1]	Heavy smokers[2]
	<1	≥1
Number of subjects(%)	37 (41)	53 (59)
Number of cigarette smoked(cigarettes/day)	2.9±0.3	10.6±0.7
Duration of smoking(year)	2.1±0.1	3.6±0.2
Average packyear	0.3±0.0	1.9±0.1

Values are mean±SE.
1) Light smokers indicate smokers whose packyears were below 1 year.
2) Heavy smokers indicate smokers whose packyears were equal to or above 1 year.

Table 2. Average daily intakes, serum levels and ratios(serum level/intake) of vitamin C and A in female adolescent students

	Non-smokers (n=85-92)	Light smokers[1] (n=30-35)	Heavy Smokers[2] (n=28-31)
Intake of vitamin C(mg)	58.2 ± 4.0	50.1 ± 5.3	58.1 ± 5.8[NS3]
Intake of vitamin A(μgRE)	281.1 ± 15.1	278.7 ± 20.7	289.6 ± 28.6
Serum vitamin C(mg/l)	12.70 ± 0.54	12.84 ± 0.64	11.41 ± 0.74
Serum retinol(mg/l)	0.91 ± 0.03	0.97 ± 0.03	0.90 ± 0.04
Serum level/intake(vitamin C)	9.53 ± 0.27	9.44 ± 0.33	8.79 ± 0.39
Serum level/intake(vitamin A)	0.37 ± 0.11	0.36 ± 0.10	0.24 ± 0.08
	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000

Values are mean±SE
1) Light smokers indicate smokers whose packyears were below 1 year.
2) Heavy smokers indicate smokers whose packyears were equal to or above 1 year.
3) NS : not significantly different among the groups at $p<0.05$ by Duncun's multiple range test.

2. Dietary intakes of vitamin C and A by smoking status

Intakes of antioxidant vitamins in female adolescents by smoking status were computed by CAN-PRO and presented in Table 2. Since the intake of vitamin E was not calculated by CAN-PRO, only intakes of vitamin C and A are presented.

The average intake of vitamin C was 58.2mg/day in non-smokers, 50.1mg/day in light smokers, and 58.1mg /day in heavy smokers. The decrease was particularly marked in individuals whose packyear was below 1 year, but significant differences among groups were not observed. As the percentage of subjects whose intakes were less than 67% of the Korean RDA was 26.2% in non-smokers, 27.3% in light smokers and 32.3% in heavy smokers, respectively, the intake of vitamin C was poor in all groups.

The average intake of vitamin A was 281.1μgR.E./day in non-smokers, 278.7μgR.E./day in light smokers, and 289.6μgR.E./day in heavy smokers. Vitamin A intakes among the groups were not significantly different. Non-smokers, light smokers and heavy smokers consume 40.2%, 39.8% and 41.4% of the Korean RDA, respectively. As the percentage of subjects whose intakes were less than 67% of the Korean RDA was 61.9% in non-smokers, 66.7% in light smokers and 70% in heavy smokers, dietary intake of vitamin A was also very poor(Fig. 1).

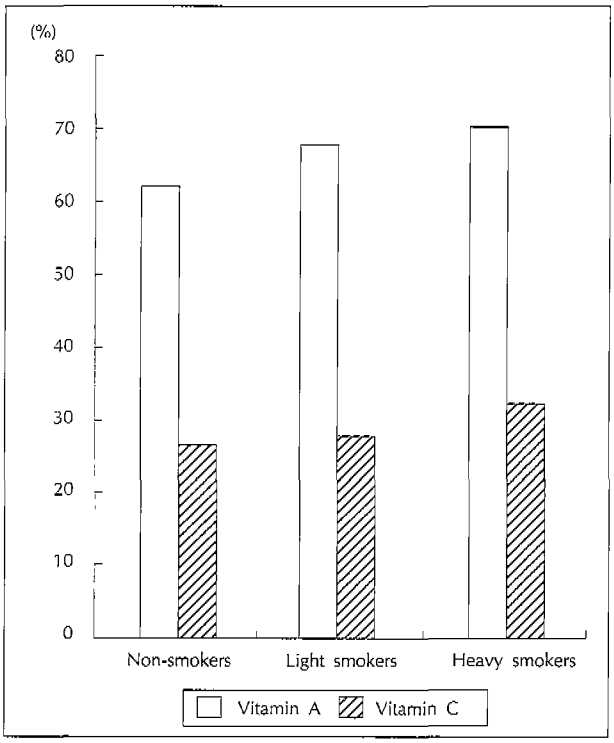

Fig. 1. Percentage of students whose intakes are below 67% of the Korean RDA of vitamin A and C in adolescent females. Light smokers indicate smokers whose packyears were below 1 year, Heavy smokers indicate smokers whose packyears were equal to or above 1 year.

Some studies reported that smokers ate less fruits and vegetables than non-smokers, leading to lower vitamin E, vitamin C and β-carotene intakes, even after adjustment for age, education and marital status(Marangon et al. 1998). Reduced intakes of vitamin C, E and β-carotene are consistently reported for smokers(Fehily et al. 1984 ; Chow et al. 1986 ; Stryker 1988), while results for retinol and total vitamin A are more varied, and may reflect differences in the social, economic class structure of the study populations(Bolton-Smith et al. 1991). But other studies (Kim & Moon 1997 ; Kim et al. 1998 ; Park & Kang 1996) reported that there were no differences in the dietary intakes of vitamins C and A between smokers and non-smokers.

3. Serum levels of vitamin C and A and their ratio per intakes by smoking status

Serum levels of vitamin C and A are shown in Table 2. Serum vitamin C level, 11.41mg/l of heavy smokers was 10% lower than that of 12.70mg/l in non-smokers or that of 12.84mg/l in light smokers although the difference was not statistically significant. The decrease of the serum vitamin C level in heavy smokers just failed to attain significance($p=0.08$). Therefore, if one compared the serum vitamin C level between non-smokers and smokers with longer smoking history, a significant difference would be observed.

Our previous reports(Kim et al. 1998 ; Kim et al. 1999) have indicated that serum vitamin C levels are lower in cigarette smokers than non-smokers. It was suggested that smoking directly lowers serum vitamin C levels(Hornig & Glatthaar 1985), although other factors including gender, age, alcohol consumption, race and BMI have also been reported to influence serum vitamin C concentrations (Baines 1982 ; Bolton-Smith et al. 1991 ; Garry et al. 1982). However, since cigarette smoking is not independent of age, sex, or alcohol use, race and BMI, the relation between smoking and vitamin C status requires evaluation controlling for these potentially confounding variables. Several previous reports have partially address-

ed these concerns by stratifying participants according to age, sex, and level of vitamin C intake(Hoefel 1977 ; Pelletier 1977 ; Ritzel & Bruppacher 1977). Within each stratum, vitamin C levels were decreased in smokers, suggesting that the relationship may indeed be independent of these factors(Schectman et al. 1989 ; Stryker et al. 1988). The reduced serum concentrations of vitamin C reported in smokers have also been shown previously(Bolton-Smith et al. 1991). Smoking is related to either decreased absorption or increased turnover of vitamin C.

Kallner et al.(1981) measured vitamin C kinetics using radio-labeled ascorbic acid and demonstrated an increased turnover of vitamin C in smokers but only small differences in absorption when compared to non-smokers. Others have reported that smoking acutely increases urinary excretion of vitamin C(Sulochana & Arunagiri 1981) also suggesting an accelerated metabolism in smokers.

The range of serum vitamin C concentration seen in persons on a normal diet is from 6 to 20mg/l ; serum vitamin C levels of 4 to 14mg/l are achieved with a dietary vitamin C intake(for adults) of ≥40mg/day(Pesce & Kaplan 1987). Criteria from the Nutrition Canada Survey (Nutrition Canada Interpretive Standard, National Survey 1973) were used to classify the risk of clinical scurvy. Respondents with serum vitamin C levels of 2mg/l or less were considered to be at high risk for clinical vitamin C deficiency(severe hypovitainosis C), while those with serum concentrations of 2 – 4mg/l were considered to be at marginal risk(marginal hypovitaminosis C)(Schectman et al. 1989).

To test the hypothesis that smoking increases the risk of hypovitaminosis C, the frequency of low serum vitamin C levels occurring in smokers was compared with nonsmokers. Of all subjects, none had serum levels of 2mg/l or less(deficiency level). The vitamin C status was generally good in both non-smokers and smokers, but 14.3% of non-smokers and 7.2% of smokers were in marginal hypovitaminosis C(2 – 4mg/l).

Schectman et al.(1989) suggested that approximately 130mg of additional dietary vitamin C daily would be required to overcome the adverse effect of cigarette smoking on serum vitamin C levels. Increasing the dietary vitamin C intake by an amount sufficient to correct the hypovitaminosis C associated with smoking should be well tolerated. Although the simplest and most direct method to increase the low serum vitamin C levels found in many smokers would be to have them stop smoking, increasing vitamin C consumption may be appropriate when cigarette cessation is unsuccessful.

The nutritional status of vitamin A can be assessed by the measurement of serum retinol. Plasma retinol con-centrations appear to be age-related, with adults having 0.32 to 0.90mg/l(Pesce & Kaplan 1987). Serum retinol is bound to its carrier protein, retinol-binding protein(RBP). Vitamin A is fat-soluble vitamin associated with many physiological functions including vision, epithelial cell maintenance, reproduction, and growth. Signs of vitamin A deficiency are usually seen at serum retinol levels less than 0.1mg/l, while those with serum concentrations between 0.1mg/dl and 0.3mg/dl were considered to be at marginal risk(Wahed et al. 1995).

Serum retinol level did not differ significantly among groups, with light smokers having higher concentrations than heavy smokers and non-smokers. The serum level of vitamin A was 0.90mg/l in non-smokers, 0.97mg/l in light smokers and 0.91mg/l in heavy smokers. Vitamin A status was generally good in both non-smokers and smokers, regardless of poor vitamin A intake.

Several studies(Bolton-Smith et al. 1991 ; Marangon et al. 1998 ; Pamuk et al. 1994) reported that there was no association between smoking and plasma retinol concentrations. Two previous studies(Comstock et al. 1988 ; Nirenberg et al. 1989), however, found a slight but significant negative association between smoking and plasma retinol levels. In our study, there was also a significant negative correlation between the amount of smoking and serum retinol level in smokers.

To adjust the difference of dietary intake of vitamin A and C, their ratios of serum levels per intakes were calculated(Table 2). There were no significant diffrences among the groups. However, the ratio of serum vitamin C level per intake was 35% lower in heavy smokers compared to non-smokers. These results might suggest that cigarette smoking has an important influence on serum vitamin C levels which occurs predominantly via a mechanism independent of decreased dietary vitamin C consumption. The lowered serum vitamin C level per intake in heavy smokers could be due to either impaired vitamin C absorption or increased turnover. On the basis of studies measuring urinary vitamin C excretion in conjunction with the administration of known vitamin C intakes, it was suggested the presence of impaired bioavai-

lability but normal turnover of vitamin C in smokers (Schectman et al. 1989). Steady-state turnover studies of Kallner et al.(1981) suggested that a higher intakes of vitamin C was required by smokers in order to maintain equivalent serum values to non-smokers.

When we examined the relationship between dietary intakes of vitamin C and A and their serum levels by smoking status, there was a significant correlation($r=0.3923$, $p<0.05$) between dietary intakes and serum levels of vitamin C only in heavy smokers and there was no significant correlation between dietary intakes and serum levels of vitamin A in all groups.

The poor association between retinol and vitamin A intakes and serum values has been reported previously(Kallner et al. 1981). It is, however, interesting to note that on partial correlation analysis, dietary β-carotene was significantly positively correlated with serum retinol in non-smokers, but not in smokers. Factors such as the concentration of retinol-binding protein, conversion rates of β-carotene to retinol, and carotene storage, all may affect circulating retinol levels(Nirenberg et al. 1989).

4. Serum levels of α-tocopherol and erythrocyte TBARS by smoking status

Serum levels of α-tocopherol, lipids and erythrocyte TBARS were presented in Table 3. The serum α-tocopherol level, 8.79mg/l in heavy smokers was about 8% lower than that of 9.53mg/l in non-smokers. The differences among groups were not significant. But the decrease in serum α-tocopherol in heavy smokers just failed to attain significance($p=0.06$).

In a similar study to ours, Stryker et al.(1988) found lower serum vitamin E levels in smokers, but the difference did not reach a significant level($p<0.05$). However, Handelman et al.(1996) reported longer cigarette smoke exposures lead to progressive losses of α-tocopherol. On the other hand, Marangon et al.(1998) reported that plasma α-tocopherol concentrations were significantly higher in ex-smokers than in non-smokers, with smokers having intermediate concentrations. Marangon et al.(1998) insisted on no effect on serum concentrations of vitamin E from smoking.

In fact, vitamin E is absorbed in conjunction with fatty acids and TG and its distribution follows that of TG or other lipids via lipoproteins. Thus serum α-tocopherol level is dependent on the serum lipid content. Serum α-tocopherol level itself is not a good indicator for vitamin status. It is generally known that serum α-tocopherol level per gram of total lipid is a better indicator for vitamin E status.

In this study, the levels of α-tocopherol/cholesterol was not significantly different among groups, but the levels of α-tocopherol/TG were significantly high in light smokers compared to the other groups ; heavy smokers had the lowest level. Such a higher level of α-tocopherol/TG in light smokers seemed to result from a significantly decreased level of TG in light smokers.

According to the study of Cho & Choi(1997), serum level of vitamin E was higher in heavy smokers(who smoke more than 20 cigarettes/day) than in light smokers(who smoke less than 20 cigarettes/day). But α-tocopherol/TG was significantly lower in heavy smokers. They reported that TG was the most correlated with serum α-tocopherol level. They recommended that α-tocopherol/TG is a better index for vitamin E status than α-tocopherol/total lipid or α-tocopherol/total cholesterol.

The decrease in plasma ascorbic acid level of smokers persisted even after statistical adjustment for the confounders(vitamin intake, alcohol consumption, age, and plasma lipid concentrations), whereas the difference in plasma α-tocopherol between ex-smokers and heavy smokers disap-

Table 3. Serum levels of α-tocopherol, lipids and erythrocyte TBARS in female adolescent students

	Nonsmokers (n=87)	Light smokers[1] (n=30-35)	Heavy smokers[2] (n=28-35)
α-Tocopherol(mg/l)	9.53 ± 0.27[a3)]	9.44 ± 0.33[a]	8.79 ± 0.39[a]
α-Tocopherol/Cholesterol(mg/g)	0.5 ± 0.0[a]	0.6 ± 0.0[a]	0.5 ± 0.0[a]
α-Tocopherol/TG(mg/g)	1.3 ± 0.1[ab]	1.5 ± 0.1[a]	1.1 ± 0.1[b]
Triglyceride(mg/l)	80.6 ± 3.6[ab]	72.5 ± 5.9[b]	88.7 ± 4.6[a]
Total Cholesterol(mg/l)	182.3 ± 3.3[a]	167.0 ± 4.0[b]	172.9 ± 5.2[ab]
TBARS(nmol/ml)	2.71 ± 0.09[a]	1.90 ± 0.04[b]	2.71 ± 0.17[a]

Values are mean±SE ; TBARS : thiobarbituric acid reactive substance

1) Light smokers indicate smokers whose packyears were below 1 year

2) Heavy smokers indicate smokers whose packyears were equal to or above 1 year

3) Means with different superscripts within a row are significantly different at $p<0.05$ by Duncun's multiple range test.

peared(Marangon et al. 1998). Several personal factors such as age, sex, BMI, socio-economic status, medication and vitamin supplementation are known to influence plasma antioxidant nutrient levels(Faruque et al. 1995). Effects of these various potentially confounding factors were minimal in our subjects as they were all young female adolescents, had similar age, body weight, BMI, blood pressure and socio-economic background(Kim et al. 1999).

Total tocopherol values above 5mg/l are generally considered nutritionally adequate. The normal range for serum total tocopherol in adults is 5 to 20mg/l. An "adequate" α-tocopherol level can be defined as 0.8mg total tocopherol/g total lipid in adults(Pesce & Kaplan 1987). Therefore the vitamin E ststus of female adolescents seemed to be generally good, regardless of smoking status.

The TBARS was measured as an index of lipid peroxidation because it is a common, simple method that provides good recovery(Cynammon 1985). The TBA test measures the concentration of malondialdehyde(MDA) produced when polyunsaturated fatty acids are degraded (Ha & Natholyn 1997). Since erythrocytes are very sensitive to oxidative damage, characterized by hemolysis, we measured erythrocyte TBARS level as a lipid peroxidation index.

Serum levels of total cholesterol, triglyceride and erythrocyte TBARS level were also presented in Table 3. Their levels were significantly lower in light smokers than in non-smokers or heavy smokers. Particularly, the TBARS level, 1.90nmol/ml in light smokers was significantly lower($p<0.05$) than that of 2.71nmol/ml in non-smokers or heavy smokers. Such a lower level of TBARS in light smokers seemed to result from the elevated α-tocopherol/TG level in light smokers. We do not know why light smokers had an elevated α-tocopherol/TG level when compared to non-smokers. But our unpublished data also showed that antioxidant enzymes activities such as superoxide dismutase and glutathione peroxidase tended to be increased in light smokers compared to non-smokers and then decreased in heavy smokers. These results might suggest that when one just starts smoking or the number of cigarette smoked per day is few, antioxidant defense system of body might be activated by cigarette smoke. But if one continue smoking and the number of cigarette smoked per day increases, antioxidant defense system of body will be decreased. These phenomenon might reflect the increased oxidative stress in heavy smokers.

5. Correlation between smoking status and biochemical indices in adolescent female smokers

Correlation between smoking status and biochemical indices in adolescent female smokers was presented in Table 4. There was a significant positive correlation($p<0.01$) between erythrocyte TBARS and packyear(Table 4, Fig 2). In addition, there was a significant positive correlation($p<0.01$) between erythrocyte TBARS and smoking quantity. This supports that the higher packyear(longer smoking), the more erythrocyte TBARS is increased. It seems that smoking elevates the oxidative stress in smokers. This result is similar to that of Ha & Natholyn(1997). Smokers may be at a high risk of erythrocyte lipid peroxidation as shown by higher hemoglobin degradation and MDA concentrations.

In our study, there was a significant negative correlation($r=-0.266$, $p<0.05$) between the amount of smoking and serum retinol level in smokers. In addition, a significant negative correlation($r=-0.278$, $p<0.05$) between TBARS level and serum retinol level was observed. But

Table 4. Correlation coefficient between smoking status and biochemical indices in female adolescent smokers

	PCKY	Q	PER	VC	VE	VA	TBARS	ECHOL	ETG
PCKY									
Q	0.894**								
PER	0.637**	0.314**							
VC	-0.073	-0.173	0.062						
VE	-0.222	-0.247	-0.063	-0.066					
VA	-0.242	-0.266*	-0.004	0.117	0.312*				
TBARS	0.416**	0.386**	0.296*	-0.180	-0.147	-0.278*			
ECHOL	0.025	0.089	0.284*	0.158	0.016	-0.008	0.008		
ETG	-0.088	-0.213	0.099	0.121	0.120	0.045	0.081	0.259**	

* : $p<0.05$, ** : $p<0.01$

PCKY : packyear ; Q : smoking quantity ; PER : smoking period ; VC serum vitamin C ; VE : serum vitamin E ; VA : serum vitamin A ; TBARS : thiobarbituric acid reactive substance ; ECHOL : α-tocopherol/total cholesterol ; ETG : α-tocopherol/TG

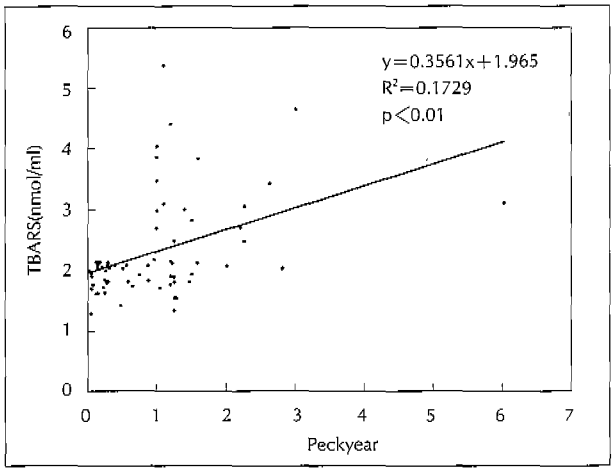

Fig. 2. The correlation between erythrocyte TBARS level and packyear in female adolescent smokers.

Bolton-Smith et al.(1991) reported that there was no significant linear trend between any serum vitamin A and the amount of smoking.

In contrast to serum retinol level, there was not a significant negative correlation between α-tocopherol level and TBARS level. But Ha & Natholyn(1997) reported α-tocopherol was apparently an effective inhibitor of TBARS formation in erythrocytes, but the mechanism by which this occurs is not known at present.

Summary and Conclusions

The purpose of this study was to investigate the effect of adolescent smoking on nutritional status of antioxidant vitamins and lipid peroxidation. We investigated dietary intakes of vitamin C and A, serum levels of vitamin C, A, E and erythrocyte TBARS levels in non-smokers and smokers who were female high school students. The results of this study are summarized as follows :

1) Since the subjects were young female adolescents, they had short packyear. Therefore, smokers were divided into 2 groups ; light smokers and heavy smokers. Mean packyear of light smokers was 0.3 year and that of heavy smokers was 1.9 year.

2) There was no significant difference in dietary intake of vitamin C by smoking. However, the serum level of vitamin C was 11.40mg/l in heavy smokers, which was about 12% lower than 12.70mg/l in non-smokers or 12.84mg/l in light smokers.

3) There was no significant difference in dietary intake of vitamin A by smoking. The serum level of vitamin A was 0.90mg/l in non-smokers, 0.97mg/l in light smokers and 0.91mg/l in heavy smokers.

4) The serum level of vitamin E was 8.79mg/l in heavy smokers, which was about 8% lower than 9.53mg/l in non-smokers. As for α-tocopherol / cholesterol or α-tocopherol / TG, there was a significant elevation in light smokers compared with the other two groups.

5) Erythrocyte TBARS level, 1.90nmol/ml in light smokers was significantly lower($p<0.05$) than that of 2.71nmol/ml in both heavy smokers and non-smokers. Such a lower level of erythrocyte TBARS seemed to be due to an elevated level of α-tocopherol/cholesterol or α- tocopherol/TG. Erythrocyte TBARS level also showed a significant positive correlation with packyear.

Since the subjects are young female adolescents, the differences between smokers and non-smokers was slight in terms of nutritional status of antioxidant vitamins and lipid peroxidation. The clinical significance of the results of the present study awaits trials on larger population groups or follow-up studies.

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