Korean J Community Nutrition 1(2) : 88~97, 1999 □ Original Article □

Serum Levels of Minerals, Ceruloplasmin, and Ferroxidase Activity in Female Adolescent Smokers*

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ABSTRACT

The purpose of this study was to investigate the nutritional status of serum minerals, ceruloplasmin, and ferroxidase activity in female adolescent students according to their smoking status. The subjects were 87 non-smokers and 88 smokers, who were female high school students. The smokers were divided into two groups by smoking status, 35 light smokers(pack-year<1) and 53 smokers (pack-year≥1). The serum concentrations of 6 minerals were determined by ICP emission spectroanalyzer. The serum concentration of ceruloplasmin and ceruloplasmin ferroxidase activity were determined. All data were statistically analyzed by SAS PC package program. The serum minerals concentrations of all subjects were in a normal range. There were significant differences in the concentrations of serum copper, zinc, magnesium, and phosphorus by smoking status while there were no significant differences in the concentrations of serum iron and manganese by smoking status. Furthermore there were no significant differences in the ceruloplasmin concentration and ferroxidase activity by smoking status. In conclusion, smoking status altered the serum levels of some minerals in healthy young women. The minerals levels in light smokers with relatively short pack-year(pack-year<1) were altered compared to those in non-smokers or smokers. This finding seemed to be consistent with the results of previously published data related to antioxidant vitamin and lipid peroxide levels. However further research is needed to clarify these findings in female adolescent smokers. (*Korean J Community Nutrition* 1(2) : 88~97, 1999)

KEY WORDS : female adolescent smokers · serum minerals · ceruloplasmin · ferroxidase · nutritional status.

Introduction

Smoking has now been described as one of the major preventable causes of mortality and it accounts for more than one-third of all deaths among middle aged in Korea (Ministry of Health and Wealfare 1999). A large number of epidemiological studies have shown a strong association between cigarette smoking and several diseases(Meng et al. 1991).

According to the recent WHO publication(1999b), estimated male smoking prevalence is 68.2% in Korea, (compared to 27.7% in the US, and 28.0% in the UK) making it the highest in the world. Estimated female smoking prevalence is 6.7%, however it is remarkably increasing. A perception that smoking is mainly a male problem is no longer valid. While only 1.4% of women smoked in 1989, that figure has risen to an estimated 6.0% in 1995(National Statistical Office 1997). Among female adolescents, the rise is remarkable : in 1997, 8.1% of female adolescents admitted to smoking, while that figure was only 2.4% in 1991(Jung 1999).

Smoking has been significantly associated with 25 causes of death(Meng et al. 1991). Particularly smoking is well-known risk factor for cardiovascular disease and lung cancer. Other health effects of smoking include nicotine addiction, dental problems, mental health effects, health-damaging behaviors, and negative effects on quality of life(US DHHS 1989). Women who smoke have at least a 10 times greater likelihood of developing lung cancer than non-smoking women. Cigarette smoking also greatly increases a woman's chance of developing cardiovascular diseases(US DHHS 1980). A woman who smokes is two to six times more likely to suffer a heart attack than a non-smoking woman, and the risk increases with the

*This research was supported by 1998 grant(KISTEP 98-N6-03-01-02) from Korean Institute of Science and Technology Evaluation and Planning

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number of cigarettes smoked each day(US DHHS 1980). The risk for cardiovascular disease also increases among young women who smoke. Thus, smoking by women is associated with deaths from heart disease and lung cancer(Fielding 1985). The risks for emphysema, bronchitis, and pneumonia are also increased among women who smoke(US DHHS 1989). Smoking may be damaging to women's reproductive health. It is associated with infertility, complications during pregnancy, and an earlier onset of menopause. The pregnant women who smoke throughout their pregnancies subject themselves, their fetuses, and newborns to significant health risks, including miscarriage, stillbirth, preterm delivery, low birth weight infants, and higher rates of infant mortality(US DHHS 1980). Exposure to environmental tobacco smoke(ETS) can cause lung cancer in otherwise healthy non-smokers and has a particularly harmful impact on children's respiratory health(WHO 1999a).

With the growing interests in the role of antioxidant nutrients in disease processes such as cardiovascular disease, cancer, and inflammatory diseases(Duthie et al. 1989 ; Gey et al. 1987), it is important to be able to assess nutritional status of antioxidant minerals in epidemiological studies. An imbalance between prooxidants and antioxidants, linked to decreased smoke-related antioxidant capacity and increased free radical generation, especially in the arterial tissue, might render smokers more prone to peroxidative stress. 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, many studies have focused on oxidative stress as a probable clinically relevant factor in cigarette smoker-related atherogenesis and cancer(Cross et al. 1998).

There is increasing evidence that oxidant mechanisms play an important role in the pathogenesis of emphysema in cigarette smokers(Janoff et al. 1983). The lungs of smokers are exposed to oxidizing substances in cigarette smoke as well as to various reactive oxygen species released by recruited lung inflammatory cells, especially alveolar macropharges and neutrophils(Babior 1978). In addition, there is evidence that the alveolar epithelial lining fluid of cigarette smokers is deficient in important antioxidants. This oxidant-antioxidant imbalance may contribute to the pathogenesis of emphysema in cigarette smokers(Janoff et al. 1983).

Several effects of copper on atherogenesis can be postulated(Klevay 1975). Although copper intake and a high ratio of copper to zinc have been traditionally considered to be protective factors(Klevay 1975), some epidemiological studies showed an association between cardiovascular mortality and copper intake or serum copper concentrations(Kok et al. 1988 ; Singh et al. 1985). An increase in risk with both high and low copper intakes has been described, as has an increase with high and low serum copper concentrations(Klevay 1975 ; Salonen et al. 1991). The ratio of Cu/Zn is used as the sensitive biological index of cancer or atherosclerosis, and diagnosis of disease and decision of symptoms. The risk of cardiovascular disease or acute heart attack is increased with the increased ratio of Cu/Zn(Salonen et al. 1991). The ratio of Cu/Zn is thought to be affected by smoking. Serum ceruloplasmin concentration is linearly related to serum antioxidant activity, a measure of its ability to inhibit lipid peroxidation(Cranfield et al. 1979 ; Galdston et al. 1984).

Another concern is the decreasing age of smoking initiation. Data revealed that in many countries, the median age of smoking initiation was under the age of 15 (WHO 1999a). This is of particular concern, since starting to smoke at younger ages increases the risk of death from a smoking-related cause. Among those who continue to smoke throughout their lives, about half can be expected to die from smoking-related cause, with half of those deaths occurring in middle age(US DHHS 1994). Smoking by adolescent women is a public problem of enormous magnitude that exacts a tremendous cost on the health of our Nation's youth today and tomorrow.

Women-specific health education and smoking cessation programmes are rare, and principally concentrate on the effects of a woman's smoking on a fetus or child. Few programmes have encouraged women to quit smoking for the sake of their own health. In addition, there is few report to investgate the effects of adolscent female smoking on health and nutritional status assessed by biochemical analysis. So, the purpose of this study was to investigate the effect of adolescent smoking on nutritional status of minerals, ceruloplasmin, and ferroxidase activity as an index of oxidative stress in female high school students.

Subjects and Methods

1. Subjects

Data were collected from 88 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 pack-year is below 1 year and smokers(n=53) whose pack-year is above 1 year. Average pack-year as concerned to 1 pack/day was calculated by the amount of smoking(pack/day) multiplied by duration of smoking(year). One pack-year means that 20 cigarettes(1 pack) per day are smoked for 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. 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. 10ml of blood samples was centrifuged at 3000rpm for 20 minutes at 4℃ to separate serum. The samples were frozen under nitrogen and stored at −80℃ until analyzed.

3. Analysis of serum minerals concentrations

The serum minerals concentrations were determined by the modified Fransson & Lonnerdal(1982) method. The serum samples were diluted ten times by 5% nitrate and analyzed using an ICP(Inductively coupled Plasma) emission spectroanalyzer(Jobin-yvon). The standards of each minerals were prepared by diluting centrified atomic absorption standard 1000ppm±1%(Fisher Scientific). The conditions for analysis are presented in Table 1.

4. Serum ceruloplasmin concentration and ferroxidase activity

1) Measurement of serum ceruloplasmin

The serum ceruloplasmin concentration was determined by measuring ρ-phenylenediamine oxidase activity by Sunderman and Nomoto(1970). 2ml of acetate buffer solution (0.1mol/l, pH 5.45) was placed in each of the glass tubes marked R(reaction) and B(blank). Exactly 0.1ml of serum was added to each tube, after which tubes were placed in a 37℃ water bath. Then 1ml of ρ-phenylenediamine dihydrochloride solution 27.6mol/l, prewarmed to 37℃, was added to each tube. After 5 minutes, the B tube was removed from the water bath. 50μl of sodium azide was immediately added and vortexed. At exactly 30 minutes, the R tube was removed and the same procedure followed. The absorbances of two tubes are measured at 530nm using a spectrophotometer(Kontron, Uvikon 930).

The concentration of ceruloplasmin was calculated by the following formula.

$$\text{Ceruloplasmin}(g/l) = 0.752(A_R - A_B)$$

$A_R/A_B \Rightarrow$ Absorbances of samples marked as R(reaction), B(blank)

2) Measurement of serum ceruloplasmin ferroxidase activity

The serum ceruloplasmin ferroxidase activity was determined by measuring ferroxidase activity using o-diani-

Table 1. Condition of ICP-spectrophotometer

	Cu	Zn	P	Mg	Fe	Mn
Analytic line(nm)	324.754	213.856	214.914	285.213	238.204	257.61
Radio frequence-output	1.2KW					
Reflective power	5.0KW					
Plasma height	14nm					
Coolant gas(Argon)	16psi(120Kpa)					
Carrier gas(Argon)	50psi(340Kpa)					
Sample uptake	1.8ml/min					
Integration time	10sec					
photo multipler tube volts	1.0KV					
Neubulizer	G.M.K-neubulizer					

sidine dihydrochloride(Schosinsky et al. 1974). In brief, 0.75ml of a sodium acetate buffer(pH 5.0) was placed in each of two glass tubes marked 5 minutes and 15 minutes. Exactly 0.05ml serum was added to each tube, after which tubes were placed in a 30℃ water bath for 5 minutes to allow temperature equilibration. Then 0.2ml of o-dianisidine dihydrochloride reagent(Sigma) prewarmed to 30℃, was added to each tube. After 5 minutes, the 5 minute tube was removed from the water bath. 2ml of 9M sulfuric acid was immediately added and vortexed. At exactly 15 minutes, the 15 minute tube was removed and the same procedure followed. The purplish-red solutions were carefully pipetted into cuvettes with a 1cm light path and the absorbances were read at 540nm on a spectrophotometer(Kontron, Uvikon 930).

The oxidase activity of ceruloplasmin was expressed in international units in terms of substrate(o-dianisidine) consumed. It was calculated as follows :

Ceruloplasmin oxidase activity=$(A_{15}-A_5)\times 6.25\times 10-1U/ml$

$A_{15}/A_5 \Rightarrow$ Absorbances of samples reacted after 5 minutes and 15 minutes

5. Statistical analysis

All data were expressed as mean±SE. The significant differences among groups were analyzed by ANOVA test, and then mean of each group was compared by Duncan's multiple range test. Correlations between smoking status and serum parameters were analyzed by Pearson's Correlation test. Statistically significant differences were accepted at $p<0.05$. SAS-PC software was used for all the analyses.

Results and Discussion

1. The concentrations of serum minerals by smoking status

The serum levels of 6 minerals were measured in female adolescent students(Table 2). There were significant differences in the concentrations of serum copper, zinc, magnesium, and phosphorus by smoking status. However, there were no significant differences in the concentrations of serum iron, and manganese by smoking status. The ranges of these minerals were covered in the normal ranges in Koreans as suggested by Lee et al.(1998), but were lower than those of adults aged over 20 years. They were slightly higher than the ranges reported by Olivieri et al.(1994).

Copper is involved in the function of several enzymes (Olivares & Uauy 1996), and required for infant growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol metabolism, myocardial contractility, glucose metabolism, and brain development(Uauy et al. 1998). Measurement of serum copper and ceruloplasmin concentrations are currently used to evaluate copper status(Milne 1994). These indexes are less sensitive to marginal copper deficiency, especially if the deficiency only recently appeared(Milne 1994). However, concentrations of these laboratory indexes are diminished in severe to moderate copper deficiency. The normal ranges for these indexes are 10.1-24.6μmol/L (64-156μg/dl) for serum copper and 180-400mg/L for ceruloplasmin. Serum concentrations of copper and ceruloplasmin change in relation to age and sex(Milne & Johnson 1993).

Table 2. Plasma mineral levels in female adolescent students

	Nonsmokers(N=82)	Light smokers[1](N=23)	Smokers[2](N=35)
Cu(μg/dl)	90.98±3.04[a3)]	75.26± 4.03[b]	94.64±3.05[a]
Zn(μg/dl)	73.65±2.76[c]	114.96±17.43[a]	93.36±4.21[b]
Cu/Zn	1.24±0.01[a]	0.65± 0.01[b]	1.01±0.01[ab]
P(mg/dl)	3.3 ±0.08[b]	4.0 ± 0.17[a]	3.1 ±0.09[b]
Mg(mg/dl)	2.1 ±0.08[b]	2.2 ± 0.08[ab]	2.4 ±0.06[a]
Fe(μg/dl)	61.0 ±0.02[ns4)]	66.1 ± 0.02	60.2 ±0.02
Mn(ng/dl)	0.17±0.00[ns]	0.03± 0.00	0.02±0.00

Values are mean±SE

1) Light smokers indicate smokers whose pack years were below 1 year

2) Smokers indicate smokers whose pack years were above 1 year

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

4) ns : not significantly different among groups within a row at $p<0.05$ by Duncan's multiple range test

N : number of subjects

The average serum copper concentration of non-smokers was 90.98±3.04μg/dl, that of light smokers was 75.26±4.03μg/dl, and that of smokers was 94.64±3.05μg/dl, respectively(Table 2). The serum copper was significantly different among the groups, with smokers having highest concentrations. It is similar to the result that serum copper in smokers was higher than that in non-smokers in the female college students in the previous study(Kim & Lee 1997). Salonen et al.(1991) reported that an increase in risk with both high and low copper intakes has been described, as has an increase with high and low serum copper concentrations. In copper deficiency, alterations of glucose and cholesterol metabolism, increased blood pressure, endothelial cell peroxidation due to a decrease in superoxide dismutase(SOD) activity, and arterial prostacyclin production may contribute to atherogenesis(Kenneth et al. 1994). Although copper intake and a high ratio of copper to zinc have been traditionally considered to be protective factors, several effects of copper on atherogenesis can be postulated(Klevay 1975). Some epidemiological studies showed an association between cardiovascular mortality and copper intake or serum copper concentrations(Kok et al. 1988 ; Singh et al. 1985).

In copper overload, the atherogenesis can be attributed to a direct effect on LDL-cholesterol oxidation by copper(Klevay 1975). LDL-cholesterol oxidation is composed of lipid peroxidation by superoxide anion originated from cell and facilitated by metallic ion such as copper and iron(Steinberg & Witztum 1990). Especially copper is reported to be a very effective pre-oxidants(Esterbauer et al. 1989).

An increased concentration of total cholesterol and low-density-lipoprotein(LDL) cholesterol and a reduced concentration of high-density-lipoprotein(HDL) cholesterol have been observed in subjects fed an experimental diet that was low in copper(Reiser et al. 1987). These are well known risk factors for the development of atherosclerosis(Klevay 1990).

In this study, the levels of serum zinc were included in the normal ranges(Lee et al. 1998). The average serum zinc concentrations of non-smokers, light smokers, and smokers were 73.65±2.76μg/dl, 114.96±17.43μg/dl, and 93.36±4.21μg/dl, respectively(Table 2). The serum zinc was significantly different among the groups, with light smokers having highest concentrations. Hambidge (1997) reported that the normal ranges of serum zinc was 75~140μg/dl. It was reported that smokers had higher serum Zn and lower serum Cu levels(Faruque et al. 1995). Kim & Lee(1997) reported that serum zinc concentration showed no significant difference in the female college students by smoking status. The differences in age in the subjects, dietary status of zinc, and the analysis methods may affect the serum levels of zinc. There's no study on a nation-wide scale about the serum levels of zinc in Korean adolescents, but only of adults and of other nations(Hambidge 1997 ; Lee et al. 1998). Therefore, it's urgent to establish the normal ranges of the serum levels of zinc in Korea.

The result that the light smokers had lowest serum copper and highest zinc level seem to be opposite to the results in female college students. This may be resulted from different life style, dietary habit, and environmental tobacco smoking. The low serum copper may reflect the low oxidative stress, since the lipid peroxide was lower in light smokers in the previous study. Also, the serum level of copper and zinc is dependent on the dietary intake, it would be clearly concluded from dietary survey of copper and zinc of subjects with long smoking history intake.

The ratio of Cu/Zn of non-smokers, light smokers, and smokers were 1.24±0.01, 0.65±0.01, and 1.01±0.01, respectively(Table 2). The ratio of Cu/Zn was significantly different among groups, with smokers having highest ratio. The ratio of Cu/Zn was 48% decreased in light smokers than in non-smokers. However, it was 55% increased than in smokers as pack-year is increased. Consequently, the ratio of Cu/Zn was higher in non-smokers than in smokers. It is not similar to the result in female college students in the previous study(Kim & Lee 1997). The actual concentrations of copper and zinc is dependent on the dietary intake status, so it is not the best index for researching the effect of smoking. So, the ratio of Cu/Zn is used as the sensitive biological index of cancer or atherosclerosis, and diagnosis of disease and decision of symptoms. The risk of cardiovascular disease or acute heart attack is increased with the increased ratio of Cu/Zn(Salonen et al. 1991). The ratio of Cu/Zn is thought to be affected by smoking status. More research about subjects with longer smoking histories could be helpful for making a clear decisions.

The average serum phosphorus concentrations of non-smokers, light smokers, and smokers were 3.3±0.08mg/dl, 4.0±0.17mg/dl, and 3.1±0.09mg/dl, respectively

(Table 2). The serum phosphorus was significantly different among the groups, with light smokers having the highest concentrations. The normal concentration of phosphorus is 2.5 – 4.8mg/dl(Henry 1974). The serum concentration of phosphorus varied according to dietary intake and age(Henry 1974). It is remarkable in the study of smoking that the serum levels of phosphorus are higher in smokers. The correlation between smoking and the serum phosphorus through an accurate dietary intake survey and physical examination is further required.

The average serum magnesium concentrations of non-smokers, light smokers, and smokers were 2.1±0.08mg/dl, 2.2±0.08mg/dl, and 2.4±0.06mg/dl, respectively (Table 2). The level of serum magnesium was significantly different among the groups, with smokers having highest concentrations. The normal range of serum magnesium is 1.7 – 2.4mg/dl(Andon et al. 1996).

The average serum iron concentrations of non-smokers, light smokers, and smokers were 61.0±0.02μg/dl, 66.1±0.02μg/dl, and 60.2±0.02μg/dl, respectively(Table 2). The serum iron was included in the normal range but was not significantly different among the groups. It is suggested that the normal range is 65 – 175μg/dl(Miles et al. 1984), and it is acceptable above the level of 40μg/dl(Ha & Na 1993).

The average serum manganese concentrations of non-smokers, light smokers, and smokers were 0.17±0.00ng/dl, 0.03±0.00ng/dl, and 0.02±0.00ng/dl, respectively(Table 2). The serum manganese was not significantly different among the groups. Manganese is an essential trace element needed for catalytic activity or activation of several enzymes(Finley 1999). It is identified to be an important biological index in the assessment of mineral status, especially for women and children(Ha & Na 1993 ; Lee et al. 1998). Finley(1999) reported that dietary manganese did not affect manganese status but iron status, as measured by serum ferritin concentration, is strongly associated with the amount of manganese absorbed from a meal by a young women. The imbalance of manganese is rare, although the study for adolescents and adults are insufficient.

2. The serum levels of ceruloplasmin and ferroxidase activity by smoking status

The serum level of ceruloplasmin and ceruloplasmin ferroxidase activity are presented in Table 3. The average serum ceruloplasmin concentrations of non-smokers, light smokers, and smokers were 9.63±0.01mg/dl, 9.83±0.02mg/dl, and 9.85±0.04mg/dl, respectively. The average serum specific ceruloplasmin ferroxidase activity of non-smokers, light smokers, and smokers were 1.88±0.31U/mg, 1.97±0.36U/mg, and 1.95±0.80U/mg, respectively. There were no significant differences in the serum levels of ceruloplasmin, ceruloplasmin ferroxidase activity, and specific ceruloplasmin ferroxidase activity.

Because measurement of antigenic amounts of ceruloplasmin(milligrams per deciliter) and oxidase activity(units per milliliter) were performed on the same serum samples, it was possible to calculate the oxidase activity per amount of ceruloplasmin. This ratio, expressed as units per milligram, allowed more precise determination of the specific enzymatic "activity" of serum ceruloplasmin.

Ceruloplasmin is one of the most important antioxidant proteins found in serum and alveolar epithelial lining fluid(Bell et al. 1981 ; Holter et al. 1986). Ceruloplasmin functions as a ferroxidase that oxidizes iron to the Fe^{+3} state, thereby preventing Fe^{+2}-catalyzed lipid peroxidation and cellular damage. Despite increased antigenic amounts of ceruloplasmin, cigarette smoker serum has previously been shown to exhibit significantly less antioxidant activity than non-smoker serum(Galdston et al. 1987 ; Pacht & Davis 1988). It was demonstrated that the decreased antioxidant actvity of cigarette smoker serum may

Table 3. Serum ceruloplasmin concentration and ferroxidase activity in female adolescent students

	Nonsmokers(N=42 – 53)	Light Smokers[1](N=30 – 32)	Smokers[2](n=6)
Serum ceruloplasmin concentration(mg/dl)	9.63±0.01[ns3]	9.83±0.02	9.85±0.04
Ferroxidase activity(U/ml)	0.15±0.02	0.16±0.03	0.16±0.06
Specific ferroxidase activity(U/mg)[4]	1.88±0.31	1.97±0.36	1.95±0.80

Values are mean±SE
1) Light smokers indicate smokers whose pack years were below 1 year
2) Smokers indicate smokers whose pack years were above 1 year
3) ns : not significantly different among groups within a row at $p<0.05$ by Duncan's multiple range test
4) Unit ferroxidase activity/milligram of ceruloplasmin
N : number of subjects

be explained by a decrease in ceruloplasmin ferroxidase activity. Because ceruloplasmin diffuses from the serum to the alveolar epithelial lining fluid, inactivation of this protein in the serum may be one mechanism that contributes to decreased lung antioxidant protection in smokers.

The mechanism for the decreased ceruloplasmin ferroxidase activity is unclear. It may be related to an increased exposure to oxidants since smokers are exposed to an increased burden of oxidizing substances contained in cigarette smoke or released by inflammatory cells recruited to their respiratory tract(Church & Pryor 1985). Previous investigators have demonstrated that ceruloplasmin is susceptible to oxidative attack and subsequent loss of ferroxidase activity(Winyard et al. 1984).

It has been hypothesized that the anemia associated with copper deficiency due to defective iron mobilization resulting from reduced ceruloplasmin activity(Danks 1988). This enzyme, by its ferroxidase action, is fundamental for the transformation of Fe^{+2} to Fe^{+3}, a step indispensable for the incorporation of iron into circulating transferrin (Frieden 1982). The reduction of ceruloplasmin may determine that the iron remains trapped in the reticuloendothelial system and is therefore not available for erythropoiesis.

Studies by one group in which the enzymatic activity and concentration of ceruloplasmin were measured showed that in copper deficient subjects, the enzymatic activity of ceruloplasmin is reduced and the ceruloplasmin concentration is conserved(Milne & Johnson 1993). Therefore, the ratio of enzymatic activity to concentration of ceruloplasmin may be a better indicator of copper status, with the additional advantage that such an index is not influenced by factors such as hormones and sex(Milne & Johnson 1993).

In this study, the serum ceruloplasmin, ferroxidase activity, and ferroxidase activity was not significantly different among the groups. The previous study of female college students(Kim & Lee 1997) showed that concentrations of copper and ceruloplasmin were significantly higher but the specific ceruloplasmin ferroxidase activity were significantly lower in the smokers. Such a different result of these two study might be due to smokers' age, average pack-year of smokers, smoking habit or lack of sample size of smokers in this study. Several studies reported that smoking does not affect serum ceruloplasmin concentration(Knekt et al. 1992 ; Mongiat et al. 1992 ; Park 1995). In addition, Pacht & Davis(1988) reported the serum levels of ceruloplasmin was increased in smokers but female smokers did not significantly differ from female non-smokers. This point might support that smoking status did not affect the serum level of ceruloplasmin and ferroxidase in female adolescents. It may be resulted from the subjects' smoking habit not inhaling to the respiratory tract. Furthermore, such results seem to be consistent with the result that smoking in female adolescents did not much influence the serum levels of antioxidant vitamins. In spite of lower lipid peroxide level, which is the index of oxidative stress, in light smokers in the previous study(Kim et al. 1999), the subjects being in healthy growth spurt resulted to the similar serum levels of ceruloplasmin and ferroxidase activity in smokers and non-smokers. Nevertheless, This study did not examine dietary intake and other potential confounding factors, more accurate study with a large sample size is recommended for definitive conclusions.

3. The correlation among biochemical indices

The correlation among biochemical indices in female adolescents is presented in Table 4.

In all of the subjects, the factor such as pack-year showed a significant positive relation to the quantity of cigarettes consumed, duration of smoking, and serum levels of magnesium. The serum level of copper showed a significant relation to the quantity of cigarettes consumed, duration of smoking. The serum magnesium and zinc showed a significant positive relation.

In smokers, the quantity of cigarettes consumed showed a significant relation to the serum level of copper. It was estimated that the smokers who smokes more may be in the increased serum copper.

It was suggested that the specific enzymatic activity of ceruloplasmin, defined as the ratio of the enzyme activity to the immunoreactive protein, is a better copper status than either the enzyme activity or immunoreactive protein alone(Milne 1994). The specific enzymatic activity of ceruloplasmin is sensitive to copper status(Milne et al. 1988) and is not affected by age, sex, or hormone use (Milne & Johnson 1993). However, there is no siginificant correlation between copper and ceruloplasmin in this study(Table 4). Ceruloplasmin protein binds 90% of the copper in plasma and the serum level of copper is affected by various factors such as diurnal variation, cancer,

Table 4. Correlations among biochemical indices in female adolescent students

	PCKY	Q	PER	Cu	Fe	Mn	Mg	Zn	P	Cer	Fer
PCKY											
Q	0.894**										
PER	0.636**	0.312**									
Cu	0.135	0.486**	0.108								
Fe	-0.045	-0.241	-0.139	-0.005							
Mn	-0.082	-0.245	-0.106	0.084	-0.048						
Mg	0.246**	0.215	0.067	0.115	-0.569**	-0.151					
Zn	0.074	-0.218	-0.203	-0.103	0.393**	-0.069	0.508**				
P	-0.079	-0.373**	-0.134	0.086	-0.101	-0.104	-0.011	0.025			
Cer	0.080	0.203	-0.000	0.187	-0.060	-0.001	-0.086	0.084	-0.080		
Fer	0.092	-0.036	-0.133	-0.094	0.075	0.181	0.191	0.275	-0.059	-0.113	

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

PCKY : packyear, Q : smoking quantity, PER : smoking period, Cu : copper, Fe : Iron, Mn : Manganese, Mg : Magnesium, Zn : zinc, Cer : ceruloplasmin, Fer : ceruloplasmin ferroxidase

and inflammation except dietary intake. Since oxygen radical and superoxide ion facilitate to unbind of copper ion from the ceruloplasmin and albumin, smoking can increase serum copper level as well as the copper binding ceruloplasmin. It is not known why serum copper level is not positively correlated with serum ceruloplasmin level in this study. Further studies is needed for this explanation.

Summary and Conclusions

The purpose of this study was to investigate the effect of adolescent smoking on the nutritional status of serum minerals, ceruloplasmin, and ferroxidase activity. The results of this study are summarized as follows :

1) There were significant differences in the concentrations of serum copper, zinc, Cu/Zn ratio by smoking status. The average serum copper concentration in light smokers was 75.26±4.03μg/dl, which was significantly lower than that of 90.98±3.04μg/dl in non-smokers or that of 94.64±3.05μg/dl in smokers. However the average serum zinc concentrations in light smokers was 114.96±17.43μg/dl, which was significantly higher than that of 73.65±2.76μg/dl in non-smokers or that of 93.36±4.21μg/dl in smokers. Consequently Cu/Zn ratio was significantly lowest in light smokers.

2) There were significant differences in the concentrations of serum magnesium and phosphorus by smoking status. The average serum phosphorus concentrations were significantly different among groups with light smokers having higher concentrations of 4.0±0.17mg/dl than non-smokers of 3.3±0.08mg/dl, and smokers of 3.1±0.09mg/dl. The average serum magnesium concentrations of non-smokers, light smokers, and smokers were 2.1±0.08mg/dl, 2.2±0.08mg/dl, and 2.4±0.06mg/dl, respectively.

3) However, there were no significant differences in the concentrations of serum iron and manganese by smoking status.

4) There were no significant differences in the ceruloplasmin concentration and specific ceruloplasmin ferroxidase activity by smoking status.

Some of the serum mineral levels of light smokers whose pack-year is below 1 year seemed to be significantly changed compared to those of non-smokers or smokers. This trend appeared to be associated with the changes of lipid peroxide value or antioxidant defense systems observed in the previous study. Though dietary intakes of minerals were not estimated, there were no significant differences in dietary intake of other nutrients by smoking status in the previous study. Overall results of this study might suggest that antioxidant mechanism and related factors would be more stimulated in light smokers than in smokers. However further study is needed to elucidate what tell us such changes of serum mineral levels in adolescent female smokers.

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