Antioxidant Vitamins and Lipid Peroxidation in Patients with Cervical Intraepithelial Neoplasia

The purpose of this study was to investigate the implications of dietary intake and the level of plasma antioxidant, lipid peroxidation, and antioxidant capacity in Korean women with cervical intraepithelial neoplasia (CIN). From October 2002 to March 2003, 58 patients diagnosed with CIN (confirmed with colposcopy directed biopsy) and 86 patients without any cervical disease as control group were enrolled in the study at the Department of Gynecology cancer center at Samsung Cheil Hospital. The intake of antioxidant vitamins in both groups exceeded the amount recommended by the Korea RDA, 7th edition. The plasma concentration of Vitamin C was significantly lower in the CIN group (0.36 mg/dL) than in the control group (0.48 mg/dL) \( (p<0.05) \). The two groups showed similar plasma concentrations of \( \beta \)-carotene, \( \alpha \)-tocopherol, and retinol. The average concentration of malondialdehydes in the CIN group, 7.23 mmol/mL, was significantly higher than in the control group, 5.18 mmol/mL \( (p<0.01) \). The total radical trapping antioxidant potential concentration of plasma was significantly higher in the CIN group (1.15 mM) than in the control group (1.25 mM) \( (p<0.05) \). These results suggest that there is a possible correlation between cervical intraepithelial neoplastic processes and changes in the plasma antioxidative system.

Key Words: Cervical Intraepithelial Neoplasia; Antioxidants; Vitamins; Lipid Peroxidation

INTRODUCTION

Cervical cancer is a critical healthcare problem throughout the world and is the foremost common cancer in women, ranking first in Korea when cases with carcinoma in situ are included (1-3). Invasive cervical cancers were once a leading cause of cancer-related deaths in the United States; however, they are now relatively uncommon. This change was attributed to the adoption of organized cytological screening (4).

Important epidemiological risk factors that contribute in the development of cervical intraepithelial neoplasia (CIN) and invasive cancer of the cervix are identified as follows: first sexual intercourse at early age, use of oral contraceptive pills, sexual promiscuity, cigarette smoking, and human papillomavirus (HPV) infection (4, 5). Many epidemiological studies and laboratory investigations revealed that nutritional factors may play an important role in the development and progression of cervical cancer. Demonstrating the relative importance of various nutrients and dietary constituents associated with cancer risk presents many challenges because of potential interaction between these factors and other etiologic factors.

Reactive oxygen species (ROS) produced by multiple factors have been implicated in multi-step carcinogenesis. Excess levels of reactive aldehydes such as malondialdehydes (MDA), a product of lipid peroxidation initiated by ROS, could alter the cellular function and lead to cancer formation (6-12).

However, the toxic effects of ROS are protected by the endogenous antioxidant defense system such as antioxidant vitamins, minerals, and antioxidant enzymes as well as inhibitors of the neoplastic process.

The nutritional etiology of cervical neoplasia in clude low dietary intake of vitamin C, carotenoids, vitamin E, and folate. For example, Nagata et al. (13) reported a case-control study that suggested the role of plasma \( \beta \)-carotene in preventing cervical cancer in Japan.

Many epidemiological and analytical studies have been conducted to investigate the relationship between antioxidant nutrients and cervical cancer (14-17). However, clinical studies in this field are very limited in Korea. The only study in the literature was a report by Kim et al. (18) on the relationship between oxidative stress increase and changes in the antioxidant system in Korean women.

The purpose of this study was to investigate the implications of antioxidant vitamin dietary intakes, plasma antioxidant vitamin (retinol, \( \beta \)-carotene, \( \alpha \)-tocopherol, and vitamin C) levels, lipid peroxidation, and antioxidant capacity in Korean women with CIN.
Materials and Methods

From October 2002 to March 2003, 58 women diagnosed with CIN (CIN group) by colposcopy-directed biopsies (performed at the Department of Gynecology cancer center at Samsung Cheil Hospital) and 86 women without any cervical disease (control group) were enrolled in this study.

Clinical characteristics and nutrient intake

The general characteristics such as weight, height, education, and life style and obstetric and gynecological characteristics such as age of menarche, menopausal status, number of pregnancies, and number of deliveries were obtained through questionnaires and medical records.

The foods intake was assessed through semi-quantitative frequency questionnaires. Nutrient analyses were carried out by using a computerized system (Computer Aided Nutritional analysis program version 2.0, 2002, Korea Nutrition Society).

Plasma antioxidant vitamin concentrations

Samples were processed under subdued or gold light. Plasma retinol, β-carotene, and α-tocopherol levels were measured by using high-pressure liquid chromatography (HPLC) as described by Bieri et al. (19). Operational conditions are shown in Table 1.

Briefly, plasma was extracted with ethanol and n-hexane containing 0.005% β-hydroxy-toluene. After centrifugation, the entire hexane layer was separated (syringe filter, 0.45 μm membrane) and evaporated under nitrogen stream. Vitamin C levels were measured by HPLC according to 2,4-dinitrophenylhydrazine methods.

Plasma total antioxidant capacity

Total radical trapping antioxidant potential (TRAP) in terms of total antioxidant capacity (TAC) of plasma was determined by the inhibition assay method described by Rice-Evans and Miller (20, 21). The assay was based on the reaction of 2,2-azino-di-(3-ethylbenzthiazoline 6-sulfonate; ABTS) with a catalyst (metmyoglobin) and H2O2 produced radical cation ABTS⁺:

\[ \text{HX-Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{X-[Fe}^{4+} = \text{O}] + \text{H}_2\text{O}. \]

ABTS⁺ + X-[Fe⁴⁺ = O] + ABTS⁺ + ABTS⁺

where HX-Fe³⁺ is metmyoglobin. The radical cation has relatively stable blue-green color, which absorbs at 734 nm of UV/VIS spectrophotometer.

TRAP levels were calculated by using Trolox calibration curve.

Plasma lipid peroxidation

Plasma lipid peroxidation (malondialdehyde; MDA as an indicator) was assayed by the fluorometric method described by Buckingham (22).
Statistical analysis

The Statistical Analysis System (SAS 8.0) for Windows was used for statistical analysis. One t-test sample was used to compare the mean difference of nutrient between the CIN group and the control group.

RESULTS

Clinical characteristics of study subjects

Table 2 shows the general characteristics and obstetrical history of CIN and control groups. The average age of the control group was 49.4 yr, which was significantly higher than the CIN group, that of 35.7 yr (p<0.001). Therefore, all the results were presented after adjustment of the age.

The average BMI (body mass index) in the CIN group, 21.5 kg/m², was significantly lower than the control group, 22.9 kg/m² (p<0.005). There was no significant difference in gender, education, marital status, drinking, smoking, and physical exercise, except for age.

The average plasma retinol concentration in the CIN group was 35.7 yr (p<0.005). There was no significant difference in menopause; 36.1% of the subjects in the CIN group and 35.7% in the control group did not experience menopause. However, there was a significant difference in menarche between two groups. However, there was a significant difference in menopause; 36.1% of the subjects in the CIN group and 35.7% in the control group did not experience menopause. However, there was a significant difference in menarche between two groups.

Without age adjustment, the intakes of total protein, animal protein, fat, retinol, and niacin in the CIN group were significantly higher than in the control group, while other nutrient intakes showed no difference. After age adjustment, there was no statistically significant difference in nutrient intakes between the two groups.

Mineral and antioxidant vitamin intakes in both CIN and control groups exceeded the Recommended Dietary Allowances for Korea (7th edition, 2002). The fat calorie ratios, 32.4% in the CIN group and 30.5% in the control group, were higher than the recommended lipid ratio of 20-25%; and the ratio of animal fat to plant fat, 1.45-1.60:1, was relatively high.

Table 3. Macronutrients and mineral intake of control and CIN groups

| Nutrients | Control (n=86) | CIN (n=58) | Crude p value | Adjusted p value |
|-----------|--------------|-----------|--------------|-----------------|
| Energy (kcal) | 1,671.9±489.4* | 1,865.3±706.7 | 0.073 | 0.388 |
| Carbohydrate (g) | 219±68.5 | 229±479.8 | 0.488 | 0.140 |
| Protein (g) | 84.5±22.9 | 95.8±34.6 | 0.031 | 0.796 |
| Animal (g) | 47.9±15.1 | 58.8±21.6 | 0.001 | 0.491 |
| Plant (g) | 36.6±10.0 | 37.0±14.3 | 0.867 | 0.115 |
| Fat (g) | 56.7±18.7 | 67.2±25.5 | 0.009 | 0.961 |
| Animal (g) | 33.6±11.1 | 41.2±15.9 | 0.002 | 0.620 |
| Plant (g) | 23.1±9.2 | 25.9±10.7 | 0.095 | 0.451 |
| Calcium (mg) | 615.5±178.6 | 631.7±229.2 | 0.656 | 0.500 |
| Phosphorus (mg) | 1,205.0±302.0 | 1,278.3±480.3 | 0.305 | 0.474 |
| Na (mg) | 5,627.1±1,619.9 | 6,314.3±2,372.1 | 0.057 | 0.925 |
| K (mg) | 3,543.0±923.5 | 3,628.8±1,375.5 | 0.679 | 0.306 |
| Fe (mg) | 16.0±4.3 | 17.0±6.7 | 0.275 | 0.677 |
| Zn (mg) | 8.8±2.3 | 9.7±3.5 | 0.013 | 0.501 |
| Vitamin A (µgRE) | 701.8±241.6 | 770.1±282.5 | 0.123 | 0.922 |
| Retinol (µg) | 102.4±41.8 | 124.9±54.7 | 0.010 | 0.624 |
| β-Carotene (µg) | 3,059.9±1,153.9 | 3,093.4±988.4 | 0.972 | 0.151 |
| Vitamin B1 (mg) | 1.3±0.4 | 1.5±0.5 | 0.108 | 0.339 |
| Vitamin B2 (mg) | 1.5±0.4 | 1.6±0.6 | 0.095 | 0.740 |
| Vitamin B6 (mg) | 3.0±0.8 | 3.1±1.2 | 0.334 | 0.386 |
| Niacin (mg) | 17.7±5.0 | 20.6±7.6 | 0.011 | 0.922 |
| Vitamin C (mg) | 169.5±64.1 | 162.5±72.5 | 0.542 | 0.100 |
| Folate (µg) | 291.9±77.1 | 305.6±113.3 | 0.425 | 0.678 |
| Vitamin E (µg) | 12.5±4.8 | 14.0±6.2 | 0.612 | 0.563 |

*Mean±S.D.

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The CIN group as well. Education, marital status, drinking, and smoking habits were similar in two groups. The control group, although older in average than the CIN group, performed more regular physical exercises than the CIN group (only 27.6% doing regular physical exercises).

Nutrient intakes

Table 3 shows the average nutrient intakes of the CIN group and control group. The energy intake in the CIN group (1,865.3 kcal) was higher than in the control group (1,671.9 kcal); however, the difference was not statistically significant.

Without age adjustment, the intake of total protein, animal protein, fat, retinol, and niacin in the CIN group were significantly higher than in the control group, while other nutrient intakes showed no difference. After age adjustment, there was no statistically significant difference in nutrient intakes between the two groups.

Mineral and antioxidant vitamin intakes in both CIN and control groups exceeded the Recommended Dietary Allowances for Korea (7th edition, 2002). The fat calorie ratios, 32.4% in the CIN group and 30.5% in the control group, were higher than the recommended lipid ratio of 20-25%; and the ratio of animal fat to plant fat, 1.45-1.60:1, was relatively high.

Plasma levels of antioxidant vitamins

Plasma antioxidant vitamin concentrations in CIN and control groups are shown in Table 4. The average vitamin C concentration in the control group was 0.48 mg/dL, which is significantly higher than 0.36 mg/dL in the CIN group (p<0.01). Both CIN and control groups showed similar average plasma concentrations of β-carotene, 40.98 µg/dL and 40.57 µg/dL, respectively. The average α-tocopherol levels of CIN and control groups were 7.31 µg/mL and 7.41 µg/mL, respectively. The average plasma retinol concentration in the control group was 57.85 µg/dL, slightly higher than 54.36 µg/dL in the CIN group, but without statistical significance.

Table 4. Plasma antioxidant vitamin levels in control and CIN groups

| Vitamin C (mg/dL) | 0.48±0.36* | 0.36±0.20 | 0.009 | 0.008 |
| β-Carotene (µg/dL) | 40.98±25.74 | 40.57±15.22 | 0.906 | 0.277 |
| α-Tocopherol (µg/mL) | 7.31±3.61 | 7.41±2.09 | 0.823 | 0.557 |
| Retinol (µg/mL) | 57.85±31.73 | 54.36±18.94 | 0.411 | 0.630 |

*Mean±S.D.
Table 5. Plasma concentrations of malonaldehyde (MDA) and total antioxidant capacity (TAC) in control and CIN groups

|                | Control (n=86) | CIN (n=58) | Crude p value | Age adjusted p value |
|----------------|---------------|------------|---------------|----------------------|
| MDA (mmol/mL)  | 5.18±2.58*    | 7.23±3.42  | <0.001        | 0.002                |
|                | (0.94-9.17)   | (2.69-14.63) |              |                      |
| TAC (nM)       | 1.25±0.15     | 1.15±0.17  | <0.001        | 0.011                |
|                | (0.71-1.58)   | (0.81-1.50) |              |                      |

*Mean ± S.D.

**Plasma levels of lipid peroxidation and total antioxidant capacity**

We measured the lipid peroxidation caused by oxidant stress in terms of plasma malondialdehydes (MDA) concentration, the peroxidant. The average plasma concentration of MDA in the CIN group was 7.23 mM/mL, which was significantly higher than 5.18 mM/mL in the control group (p<0.01) (Table 5).

On the contrary, the average antioxidant capacity of plasma was 1.25 mM in the control group, significantly higher than 1.15 mM in the CIN group (p<0.05).

**DISCUSSION**

The BMI in the CIN group was lower than that in the control group possibly due to the higher average age of the control group (49.4 yr). HPV infection and smoking (non-dietary factors of cervical cancer) were suggested (4) as risk factors, but only 7% of the control group and 12% of the CIN group were smokers in our study. There was no significant difference in smoking habit between the two groups.

Balanced total fat intake is crucial since the increased intake of animal fat leads to increased lipid intake, which results in the increase of saturated fat and energy density. Dietary fat was the most widely studied dietary factor in relation to the onset of cancer. Based on the amount and the kind of lipid fatty acid, lipid intake has different effects on the onset of cancer. A retrospective study (23) showed that the increase in animal fat intake increased the risk of colon cancer and breast cancer.

Studies on the relationship between lipid intake and cervical cancer have not yet been reported. However, unbalanced fat intake could change the fatty acid composition of cell membrane, which can eventually cause abnormal membrane fluidity and permeability and affect the roles of membrane enzymes and receptors, potentially prompting the growth of cancer cells. Therefore, suppressing lipid intake and increasing antioxidant nutrient intake that could prevent lipid peroxidation are important.

The intake of antioxidant vitamins is known to have preventive effects on cervical cancer (15). However, the dosage necessary to produce the same level of effectiveness between simple manipulated antioxidant material and antioxidants in more complicated form, such as foods, is still controversial.

In recent studies, the plasma carotenoid concentration in women with cervical cancer decreased significantly compared to the control group in Korea (18). All of the CIN and control subjects enrolled in this study showed inadequate vitamin C status: 0.48 mg/dL in the control group and 0.36 mg/dL in the CIN group. It is difficult to assess vitamin C retrospectively because the analysis process requires sensitive examination methods and the sample storage needs sophisticated techniques.

Previous studies (8, 10) reported that the average plasma vitamin C concentration was lower in cervical cancer patients than in control subjects. These studies also observed significantly lower plasma vitamin C concentrations in women with uterine cervix inflammation, although there was no significant variation in plasma vitamin C concentrations according to the stage of tumor. In addition, the plasma vitamin C concentration in the CIN group was slightly lower than in the control group in a recent study conducted in Korea (18). It can be concluded that vitamin C plays beneficial roles in early immunity and/or antioxidant reaction in cervical tissue.

Lee et al. (24) reported that the average plasma β-carotene concentration in the general Korean adult population was 0.23 µg/mL, while it was 0.47 µg/mL in female adults. The average plasma β-carotene concentration in this study was 40 µg/dL with no significant difference between the two groups. In a study by Kim et al. (18), the average plasma β-carotene concentration in cervical neoplasia patients was 36.20 µg/dL, lower than 49.59 µg/dL in control group. Melissa et al. (25) also reported that the average plasma β-carotene concentration in the CIN group was usually lower than in the control group. Moreover, the concentration was significantly lower in women with a more advanced tumor status. Since it is known that β-carotene affects cell division processes and prevents abnormal cell development, an adequate amount of β-carotene is important (6) in maintaining healthy tissue.

The average plasma α-tocopherol concentration in women with cervical neoplasia was 6.28 µg/mL (18), significantly lower than 7.62 µg/mL in this control group. In other case-control study (9), women with cervical neoplasia had a lower plasma α-tocopherol concentration than control women. However, a study (26) performed in Latin America did not found the effect of α-tocopherol on cervical cancer, nor a cohort study (27) conducted in Washington D.C. of the United States. In this study, the plasma α-tocopherol concentration was not different CIN and control groups.

The average plasma retinol concentrations in this study were lower than the average concentration (63.1 g/mL) previously reported in Korean female adults (28). Since the role of vitamin A in cellular division is well known, the effects of retinol on the prevention and treatment of cervical neoplasia have drawn great attention (25, 26, 28, 29). However,
case-control studies found no significant relationship between cervical neoplasia and total vitamin A intake (25, 26). Moreover, cohort study (30) found no effects of plasma retinol on cervical cancer.

In this study, the MDA concentration was a following lower in the CIN group than in the control group. This suggests degeneration in the antioxidative system and overproduction of peroxidant. Kim et al. (18) also reported that the plasma MDA concentration in women with CIN was higher (5.96 mmol/mL) than in control subjects (3.02 mmol/mL).

The concentration of each antioxidative nutrient can be measured by different methods, but recently developed TRAP by Miller et al. (21) has been widely used to analyze total antioxidative capacity. Kim et al. (31) reported that plasma TRAP concentration in patients with CIN (1.16 mM) was significantly higher than in cancer patients (1.03 mM). Our data support this finding. In terms of plasma MDA and TRAP concentrations, the index of total antioxidative effect, changes in antioxidative system and oxidative stress are thought to affect the development and progression of CIN.

While there are a number of previous studies on analysis of plasma concentration of vitamins with antioxidative effects, reports on peroxidant accumulation or total antioxidant capacity are limited. Furthermore, clinical nutritional studies on cervical neoplasia in Korean women have been rare.

Systematically cooperative researches between clinical area of Gynecology and Obstetrics and clinical nutrition are needed to elucidate the role of antioxidative nutrients on nutritional condition of the body and the development and progression of cervical neoplasia, and ultimately to establish nutritional guidelines to prevent CIN and cancer.

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