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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 β-carotene, α-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*

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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. Excessive 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 β-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, β-carotene, α-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-dinitrophenyhydrazine methods.

Table 1. Operational conditions of HPLC for the determination of retinol, β-carotene, α-tocopherol, and Vitamin C concentrations.

Instrument	Waters 2690 Sperations Module Hewlett Packard Vectra 500 series Millennium 2010 LC (version 2.15.01)	
Column	Supelcosil LC-18, 25 cm × 4.6 mm, 5 μm (Sigma-Aldrich Co. U.S.A.)	
Detector	retinol & α-tocopherol	UV (280 nm)
	β-carotene	UV (450 nm)
	Vitamin C	UV (254 nm)
Mobile phase	retinol & α-tocopherol: Methanol:Acetonitrile:H_2O=25:25:1 β-carotene: (Ethanol:Acetonitrile=1:1)+0.1 mL diethylamine Vitamin C:$KH_2PO_4$0.01M+PIC A regent 1ea	
Flow rate	retinol, α-tocopherol, β-carotene: 1.0 mL/min Vitamin C: 0.7 mL/min	
Run time	retinol & α-tocopherol: 30 min β-carotene & vitamin C: 20 min	
Injection volume	retinol & α-tocopherol: 15 μL β-carotene: 30 μL Vitamin C: 20 μL	

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 H_2O_2 produced radical cation $ABTS^+$:

$HX\text{-}Fe^{3+} + H_2O_2 \rightarrow X\text{-}[Fe^{4+}=O] + H_2O_2$

$ABTS + X\text{-}[Fe^{4+}=O] \rightarrow ABTS^+ + ABTS^+$

where $HX\text{-}Fe^{4+}$ 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).

Table 2. Clinical characteristics of the study subjects

Characteristics	Control (n=86)	CIN (n=58)
Age (yrs)	49.4±11.8*	35.7±9.3§
Weight (kg)	56.2±6.5	55.3±7.7
Height (cm)	156.7±3.9	160.2±5.1
BMI (kg/m^2)	22.9±2.9	21.5±2.7‡
Menarche (yrs)	14.8±1.6	14.6±1.6§
Menopause		
Yes	31 (36.1)†	3 (5.2)
No	55 (63.9)	55 (94.8)
Gravidity	3.4±2.7	2.8±2.0
Parity	2.0±1.4	1.2±0.9†
Education		
≤High school	64 (74.4)	34 (59.7)
≥College	22 (25.6)	23 (40.3)
Marital status		
Unmarried	4 (4.7)	6 (10.3)
Married	77 (89.5)	50 (86.2)
Divorced/Separation by death	5 (5.8)	2 (3.5)
Regular Exercise		
Yes	44 (51.2)	16 (27.6)
No	42 (48.8)	42 (72.4)
Alcohol		
Yes	43 (50.0)	32 (55.2)
No	43 (50.0)	26 (44.8)
Smoking		
Yes	6 (7.0)	7 (12.1)
No	80 (93.0)	51 (87.9)

*Mean±S.D, †Number of subjects (%), ‡Significantly different at $p<0.01$, §Significantly different at $p<0.001$.

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/m^2, was significantly lower than the control group, 22.9 kg/m^2 ($p<0.005$). There was no significant difference in age of menarche between two groups. However, there was a significant difference in menopause; 36.1% of the subjects in the control group were in menopause, which was significantly higher than 5.2% in the CIN group ($p<0.001$).

The control group had more pregnancies and deliveries than 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 intakes of total protein, animal protein, animal 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	Age adjusted *p* value
Energy	(kcal)	1,671.9±489.4*	1,865.3±706.7	0.073	0.388
Carbohydrate	(g)	219.1±68.5	229.4±97.8	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±232.9	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	0.625
β-Carotene	(μg)	3,059.9±1,153.9	3,053.4±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	0.366
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	(mg)	12.5±4.8	14.0±6.2	0.121	0.563

*Mean±S.D.

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 of in the CIN group, but without statistical significance.

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

	Control (n=86)	CIN (n=58)	Crude *p* value	Age adjusted *p* value
Vitamin C (mg/dL)	0.48±0.36* (0.02-1.42)	0.36±0.20 (0.06-0.84)	0.009	0.008
β-Carotene (μg/dL)	40.98±25.74 (14.97-119.55)	40.57±15.22 (11.77-67.19)	0.906	0.277
α-Tocopherol (μg/mL)	7.31±3.61 (1.56-18.69)	7.41±2.09 (2.20-13.90)	0.823	0.557
Retinol (μg/dL)	57.85±31.73 (4.16-140.01)	54.36±18.94 (21.64-78.56)	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* (0.94-9.17)	7.23±3.42 (2.69-14.63)	<0.001	0.002
TAC (mM)	1.25±0.15 (0.71-1.58)	1.15±0.17 (0.81-1.50)	<0.001	0.011

*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 concentration 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 μg/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) is now widely used to analyze total antioxidant 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 sudies on cervical neoplasia in Korean women have been rare.

Systemically 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.

REFERENCES

1. Parkin DM, Pisani P, Ferlay J. *Estimates of the worldwide incidence of 25 major cancers in 1900. Int J Cancer 1999; 80: 827-41.*
2. Pisani P, Park DM, Bray F, Ferlay J. *Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer 1999; 83: 18-29.*
3. Annual report on the cause of death statistics. *National Statistical Office Republic of Korea 2001.*
4. Koss LG. *Cervical (pap) smear. Cancer 1993; 71: 1046-12.*
5. Herrero R. *Epidemiology of cervical cancer. Monorgr Natl Cancer Inst 1996; 21: 1-6.*
6. Kim YT, Kim JY, Choi JS, Kim SH, Choi EK, Cho NH. *Relation between deranged antioxidant system and cervical neoplasia. Int J Gynecol Cancer 2004; 14: 889-95.*
7. Manoharan S, Kolanjiappan K, Kayalvizhi M, Sethupathy S. *Lipid peroxidation and antioxidant status in cervical cancer patients. J Biochem Mol Biophys 2002; 6: 225-7.*
8. Ahmed MI, Fayed ST, Hossein H, Tash FM. *Lipid peroxidation and antioxidant status in human cervical carcinoma. Dis Markers 1999; 15: 283-91.*
9. Manju V, Kalaivani SJ, Nalini N. *Circulating lipid peroxidation and antioxidant status in cervical cancer patients: a case-control study. Clin Biochem 2002; 35: 621-5.*
10. Senthil K, Aranganathan S, Nalini N. *Evidence of oxidative stress in the circulation of ovarian cancer patients. Clin Chim Acta 2004; 339: 27-32.*
11. zur Hausen H. *Human pathogenic papillomaviruses. Heidelberg, Germany: Springer Verlag, 1994.*
12. Koutsky LA, Holmes KK, Critchlow CW, Stevens CE, Paavonen J, Beckmann AM, DeRouen TA, Galloway DA, Vernon D, Kiviat NB. *A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomaviris infection. N Engl J Med 1992; 327: 1272-8.*
13. Nagata C, Shimizu H, Yoshikawa H, Noda K, Nozawa S, Yajima A, Sekiya S, Sugimori H, Hirai Y, Kanazawa K, Sugase M, Kawana T. *Serum carotenoids and vitamins and risk of cervical dysplasia from a case-control study in Japan. Br J Cancer 1999; 81: 1234-7.*
14. Mackerras D, Irwig L, Simpson JM, Weisberg E, Cardona M, Webster F, Walton L, Ghersi D. *Randomized double-blind trial of beta-carotene and vitamin C in women with minor cervical abnormalities. Br J Cancer 1999; 79: 1448-53.*
15. Shannon J, Thomas DB, Ray RM, Kestin M, Koetsawang A, Koetsawang S, Chitnarong K, Kiviat N, Kuypers J. *Dietary risk factors for invasive and in-situ cervical carcinomas in Bangkok, Thailand. Cancer Causes Control 2002; 13: 691-9.*
16. Wideroff L, Potischman N, Glass AG, Greer CE, Manos MM, Scott DR, Burk RD, Sherman ME, Wacholder S, Schiffman M. *A nested case-control study of dietary factors and the risk of incident cytological abnormalities of the cervix. Nutr Cancer 1998; 30: 130-6.*
17. Head KA. *Ascorbic acid in the prevention and treatment of cancer. Altern Med Rev 1998; 3: 174-86.*
18. Kim JW, Choi EK, Lim JH, Kim YT, Kim DK, Lee-Kim YC, Kim SY, Chung HY. *Antioxidant system and oxidative stress in cervical cancer of Korean women. Korean J Obstet Gynecol 2002; 45: 145-51.*
19. Bieri JG, Tolliver TJ, Catignani GL. *Stimultaneous determination of β-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr 1979; 32: 2143-9.*
20. Rice-Evans C, Miller NJ. *Total antioxidant status in plasma and body fluids. Methods Enzymol 1994; 234: 279-93.*
21. Miller NJ, Rice-evans C, Davies MJ, Gopinathan V, Milner A. *A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond) 1993; 84: 407-12.*
22. Buckingham KW. *Effect of dietary polyunsaturated/saturated fatty acid ratio and dietary vitamin E on lipid peroxidation in the rat. J Nutr 1985; 115: 1425-35.*
23. Kuller LH. *Dietary fat and chronic diseases: epidemiologic overview. J Am Diet Assoc 1997; 97: 9-15.*
24. Lee KY, Lee YC, Park YS, Yoon KH, Kim BS. *A study of relation between dietary vitamin A intake ane serum vitamin A level and cancer risk in Korea. Korea J Nutr 1985; 18: 301-11.*
25. Melissa AS, Ruth EP, Richard NB, Mary M, Linda van Asselt-King, Cosette MW, Thomas MB. *Serum carotenoids and risk of cervical intraepithelial neoplasia in southwestern american indian women. Cancer Epidermiology, Biomarkers & Prevention 2001; 10: 1219-22.*

26. Potischman N, Herrero R, Brinton LA, Reeves WC, Stacewicz-Sapuntzakis M, Jones CJ, Brenes MM, Tenorio F, de Britton RC, Gaitan E. *A case-control study of nutrient status and invasive cervical cancer. II. Serologic indicators. Am J Epidemiol 1991; 134: 1347-55.*
27. Batieha AM, Armenian HK, Norkus EP, Morris JS, Spate VE, Comstock GW. *Serum micronutrients and the subsequent risk of cervical cancer in a population-based nested case-control study. Cancer Epidemiol Biomarkers Prev 1993; 2: 335-9.*
28. Ziegler RG, Brinton LA, Hamman RF, Lehman HF, Levine RS, Mallin K, Norman SA, Roserthal JF, Trumble AC, Hoover RN. *Diet and the risk of invasive cervical cancer among white women in the United States. Am J Epidemiol 1990; 132: 432-45.*
29. Herrero R, Potischman N, Brinton LA, Reeves WC, Brenes MM, Tenorio F, de Britton RC, Gaitan E. *A case-control study of nutrient status and invasive cervical cancer. I. Dietary indicators. Am J Epidemiol 1991; 134: 1335-46.*
30. Batieha AM, Armenian HK, Norkus EP, Morris JS, Spate VE, Comstock GW. *Serum micronutrients and the subsequent risk of cervical cancer in a population-based nested case-control study. Cancer Epidemiol Biomarkers Prev 1993; 2: 335-9.*
31. Kim SY, Kim JW, Ko YS, Koo JE, Chung HY, Lee-Kim YC. *Changes in lipid peroxidation and antioxidant trace elements in serum of women with cervical intraepithelial neoplasia and invasive cancer. Nutr Cancer 2003; 47: 126-30.*