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Vitamin C Nutriture in Newly Diagnosed Diabetes

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Summary This study was performed to investigate the relationship between serum L-ascorbic acid, vitamin C intake, and diabetes in a nested case-control study. A cross-sectional survey of diet and health was conducted in 2,048 adults with an age of 30 y or older in Yonchon County, Korea. An oral glucose tolerance test was administered to all participants. One hundred cases of newly diagnosed diabetes were identified. Two healthy controls for each case matched with age, gender, drinking status, and smoking status were selected among the survey participants. L-Ascorbic acid levels were analyzed in fasting serum samples and one 24-h dietary recall was performed. Dietary vitamin C intake of persons with diabetes was 50.1±47.6 mg/d and that of controls was 55.1±41.1 mg/d. People with diabetes (22.3±16.8 μmol/L) have lower serum ascorbic acid levels than their controls (26.3±17.0 μmol/L) and the difference was significant by paired *t*-test ($p<0.01$). The association between diabetes and serum ascorbic acid level was still significant in non-smokers (24.2±17.8 μmol/L for the diabetes group and 29.5±16.7 μmol/L for the control group, $p<0.01$) but not in smokers (19.4±15.7 μmol/L for the diabetes group and 21.2±16.0 μmol/L for the control group). Our results suggest that diabetes and smoking interactively affect serum ascorbic acid levels. Since this population had poor nutritional status of vitamin C, further investigation of association between serum ascorbic acid level and diabetes and smoking by the level of vitamin C consumption is warranted.

Key Words vitamin C intake, serum ascorbic acid, diabetes, smoking, case-control study

Vitamin C is one of the first lines of antioxidant defense and is involved in a variety of biological processes (*1*, *2*). It has been suggested to exert beneficial effects on complications related to diabetes mellitus (DM) due to the reduction in the polyol pathway and oxidative stress following glycosylation of proteins (*3*–*5*).

Nonetheless, therapeutic effects of vitamin C supplementation to normalize serum ascorbic acid levels in DM must be assessed to determine the adverse effects associated with mega doses (*6*–*8*). There is no clear beneficial evidence of vitamin C supplementation for people with DM, which is not included in the current recommendations due to uncertainties related to long term efficacy and safety (*9*, *10*).

Several studies have reported low ascorbic acid and high dehydroascorbic acid levels in humans and animals with DM (*11*–*15*). However most previous studies on vitamin C status in people with DM have been criticized for weak methodology (*16*). Population studies have found no association of serum vitamin C levels (*17*) or vitamin C intake with DM (*18*). However, the EPIC-Norfolk study reported significant inverse associations between plasma vitamin C levels and HbA_1c (*19*) and development of DM (*19*, *20*).

DM has increased dramatically in Korea in recent years. The prevalence of DM in Korea increased by five to six fold over the past years while the increase was about two fold in USA during the same period (*21*). In the preliminary report of 2007 Korean National Health and Nutrition Examination Survey, the prevalence of DM among adults with 30 y of age or older was estimated to be 9.5% (*22*). However, there has been no population study on the relationship between serum vitamin C levels and DM in Korea or countries outside North America and Europe. This study was conducted to investigate associations between DM and serum ascorbic acid levels in a nested case-control study.

MATERIALS AND METHODS

Baseline survey. A cross-sectional diet and health survey was conducted in Yonchon County, Korea with 2,048 voluntary participants with an age of 30 y or older. Informed consent was obtained from all participants. The details of the study have been described in previous reports (*23*, *24*). In addition to completing a detailed health and life style questionnaire, participants were measured for height and weight and given a clinical examination. BMI was calculated as weight/height^2 (kg/m^2). Smoking history was determined by yes/no responses to the questions: "Have you ever smoked cigarettes?"; "Do you smoke cigarettes now?"; and "How many cigarettes do (did) you smoke?" Drinking history was similarly determined by questions, such as "How

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Table 1. Selected characteristics and vitamin C nutriture of subjects with newly diagnosed diabetes and normal controls.

Characteristics	Diabetes (n=100)	Controls (n=200)
Age[1] (y)	57.6±13.4	57.4±12.8
Male (%)	66.0	66.0
Current smokers (%)	39.0	38.0
Drinkers (%)	62.0	56.5
BMI[1] (kg/m^2)	25.3±3.8**	24.3±3.2
Blood pressure (mm/Hg) [1]		
Systolic	138.4±26.2**	129.1±20.3
Diastolic	87.8±18.2**	80.3±14.6
Plasma glucose (mmol/L) [1]		
Fasting	8.43±2.57**	5.74±0.36
2-h after glucose load	12.4±4.80**	5.33±1.17
Dietary intake[1]		
Energy (kcal/d)	1,665±772	1,687±679
% Energy from protein	14.6±4.87	14.1±4.89
% Energy from fat	15.3±8.88	15.4±8.05
% Energy from carbohydrates	60.4±16.7	62.9±13.5
Vitamin C (mg/d)	50.1±47.6	55.1±41.1
Serum ascorbic acid[1] (μmol/L)	22.3±16.8*	26.3±17.0

[1] Mean±SD.
* Significantly different from controls by paired t-test (** $p<0.0001$, * $p<0.01$).

many alcoholic beverages do you drink a day?" Dietary intake data was obtained by a trained dietitian using a 1 d 24-h recall method. Subjects were asked to describe what they had eaten the previous day and to estimate portion sizes by referring to pictures and three-dimensional food models. Intakes of energy and nutrients were obtained using a computerized nutrient analysis system, DS24. An oral glucose tolerance test (OGTT) was given to participants who had no prior diagnosis of DM and the procedure was previously reported in detail (*23*).

Subject selection. Subjects of the present study were selected among the participants of the baseline survey. From the oral glucose tolerance test, 104 newly diagnosed DM cases were identified. Two controls for each DM case were selected among the participants with normal glucose tolerance matched with age, gender, smoking and drinking status. However, three cases and one control with abnormally high serum ascorbic acid concentrations were excluded later along with their matched pairs (*25*). Thus, 100 persons with DM and 200 controls were included in this study. Characteristics of these subjects are summarized in Table 1.

Assays. Serum ascorbic acid was assayed from fasting blood samples using High Performance Liquid Chromatography (HPLC). All specimens were centrifuged at 3,000 rpm within 1 h of collection. Two hundred microliters of serum extract was diluted with 800 μL of freshly prepared 5% metaphosphoric acid solution for stabilization during the storage period (*26*). The gently mixed specimens were stored at −80°C and analyzed within 6 mo. Ascorbic acid stock solution (5.678 mmol/L) was prepared by dissolving 567.8 μmol of L-ascorbic acid (Shinyo Pure Chemical Co., Ltd., Japan) in 100 mL of 5% metaphosphoric acid solution and stored at −80°C. Frozen samples were thawed and centrifuged at 3,500 ×g for 4 min before analysis. A working standard (5.678 μmol/L) was prepared by diluting the ascorbic acid stock solution with 5% metaphosphoric acid solution. The HPLC system consisted of a Young-in Model 910 solvent delivery module, Waters 440 UV detector, and a μ-Bondapak C_{18} Column. Twenty microliters of supernatant was injected into the chromatographic system and eluted with 0.01 M potassium phosphate (Merck Chemical Co., Ltd., Germany) with PIC A reagent (Waters, USA) at a flow rate of 0.7 mL/min. Under these conditions, ascorbic acid was eluted after approximately 9 min. Sample concentrations were calculated from peak areas.

Statistical analysis. The paired t-test was used to compare numeric variables between DM cases and controls. Distributions of categorical variables between the two groups were tested by a chi-square test. Serum ascorbic acid status was assessed within each DM case and control group. The cut-off values were serum ascorbic acid level <11.4 μmol/L for deficient status, 11–23 μmol/L for low status, and >23 μmol/L for adequate status (*27*). Among groups with different serum ascorbic acid status, the differences in means of numeric variables were tested by ANOVA and distributions of categorical variables were compared by the Mantel-Haenszel chi-square test. A value of α=0.05 was used for statistical significance. All statistical analyses were performed using SAS software, version 9.13.

RESULTS

People with newly diagnosed DM and their respective controls were matched with age, gender, smoking status, and drinking status (Table 1). The two groups had similar dietary energy intakes and their sources of

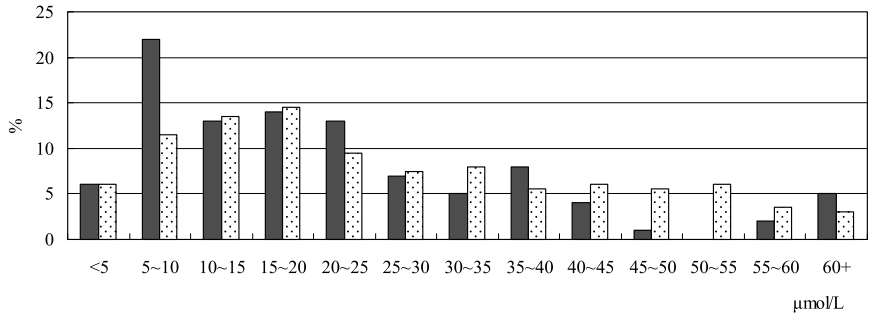

Fig. 1. Distribution of serum ascorbic acid. ■ diabetes, ⬚ control.

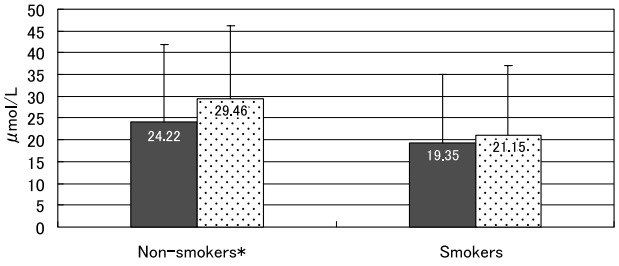

Fig. 2. Comparison of serum ascorbic acid levels between diabetes and control groups by smoking status. *Mean difference between diabetes and control groups was statistically significant by paired *t*-test ($p<0.01$). ■ diabetes, ⬚ control.

energy were proportional (Table 1).

The DM group had a slightly higher mean BMI than the controls; however, the quartile distribution was similar (data were not shown). Blood pressures and plasma glucose concentrations were significantly higher in people with DM than in their controls (Table 1). Subjects in the DM group had lower serum concentrations of ascorbic acid than their controls but the two groups had similar dietary vitamin C intakes (Table 1). Distributions of serum ascorbic acid level by DM status (diabetes and controls) are presented in Fig. 1. Distribution is more skewed to lower levels of serum vitamin C among subjects with DM. When we assessed the serum ascorbic acid status, 50% of controls had adequate status but only 39% of people with DM did. More subjects in the DM group were assessed as vitamin C deficient (30%) or low (31%) status than those in the control group (21.5 and 28.5%, respectively) with marginal significance ($p=0.0501$).

When we analyzed the influence of age, gender, smoking and drinking on the ascorbic acid status by DM status, the deficient group consisted of higher proportion of males, drinkers and older people in both the DM and control groups while smoking status affected serum ascorbic acid level only in the control group (data not shown). As shown in Fig. 2, mean serum ascorbic acid concentrations of non-smoking people with DM and the controls were 24.2±17.8 and 29.5±16.7 μmol/L, respectively. Those of smokers were 19.4±15.7 and 21.2±16.0 μmol/L, respectively. Mean difference in serum ascorbic acid concentration between non-smoking DM and control groups was 5.25±21.91 μmol/L and statistically significant ($p<0.01$). That of smoking pairs was 1.8±20.52 μmol/L.

DISCUSSION

The results of our study are generally in line with the report of a large prospective study by Harding et al. (*20*). Lower serum vitamin C levels are significantly associated with DM in both studies. However, the obvious difference is the low levels of serum vitamin C in our subjects. Mean concentration of all subjects in our study was 25.0 μmol/L, compared to the 50.5 μmol/L (in spring) and 56.2 μmol/L (in autumn) in the study by Harding et al. (*20*). In our study, only 44% of subjects were in the adequate range of serum vitamin C level. Serum vitamin C levels in our study subjects were low when compared to previous studies (*17*, *18*, *28–33*). Vitamin C intake from 1 d dietary recall in our subjects was also low. The mean intake level of our subjects was approximately 53.4 mg/d, while dietary intake of vitamin C in the study from Will et al. (*17*) was 104 mg/d. Since our survey was conducted in winter, the intake level could be lower than in summer when more fruits and vegetables are available in the rural study area. However, many subjects in our study seem to be at risk of low vitamin C status. Therefore, it seems the relationship between low serum vitamin C levels and DM covers in a wide range of vitamin C status. When we compared serum ascorbic acid by quartiles of dietary vitamin C, the mean serum ascorbic acid level of persons with diabetes was much lower than that of normal persons in the lowest quartile of vitamin C intake (≤24.09 mg/d). In the higher quartiles of vitamin C intake, the serum ascorbic acid levels were not different (data not shown). Further investigation of the relationship between DM and serum ascorbic acid concentration in different consumption levels is required.

In previous studies, men and the elderly have lower ascorbic acid concentrations than women and normal adults, respectively (*28–30*). In addition, other groups at risk for low vitamin C status include cigarette smokers and alcoholics (*30–33*). Therefore, in the present study, each control subject was selected to be matched with the age, gender, smoking and drinking status of a person with DM. As a result, there was no statistical difference in these factors between DM cases and control subjects. However, one interesting thing is smoking status seemed to modify the effect of DM on serum ascorbic

acid levels. Among smokers, DM status didn't make a difference in serum ascorbic acid levels. Smoking is well known to increase oxidative stress both directly and indirectly (*34*). At the same time, it could be noted that one of the potential mechanisms associated with DM is oxidative stress (*35*). Our result might indicate that smoking and DM interact with each other in increasing oxidants and decreasing natural antioxidants. Whether this is biologically significant needs further study.

In this study, the DM group had higher blood pressure than the control group. A large volume of literature reports the negative association of serum ascorbic acid with blood pressure (*36–39*). The researchers focused on the hypotensive action of ascorbic acid based on its biological function to explain their observed results (*37–39*). In addition, one recent study showed that ascorbic acid interfered with angiotensin activity and explained the blood pressure lowering effect of ascorbic acid (*40*). However, our result might be speculated to be due to the difference in blood pressure between the DM and control groups because high blood pressure could increase renal flow and decrease the body pool size of nutrients. A future study may need to consider this influence in the study design.

Despite the rapid increase in DM in Korea, there has been little research regarding the roles of diet in the etiology of DM. Our study showed that serum vitamin C levels of newly diagnosed DM patients are lower than those of people without DM, especially in non-smokers. In our study, subjects with DM consumed less vitamin C than their counterparts but the difference was not significant. Therefore, we were unable to address whether the low serum levels and the coexistence of DM was a cause of the disease or the consequence, while the studies by Harding et al. (*20*) show the evident causal relationship of vitamin C in the development of DM. However, it is controversial whether a high oral dose of vitamin C is beneficial in persons with DM (*41–45*) or preventing DM (*46*). Besides the protective effect of a high dose, it may be necessary to study whether low or deficient status of vitamin C leads or accelerates the development of DM. Our research has limitations: 1) it was conducted in a relatively small number of subjects, 2) dietary intake was assessed only for 1 d while usual intake is more useful to assess nutritional status. Considering the rapid increase of DM in many non-western countries, the relationship between vitamin C nutriture and the development of DM needs to be studied using larger prospective studies in these regions.

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