Lymphocyte and Plasma Vitamin C Levels in Type 2 Diabetic Patients With and Without Diabetes Complications

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Diabetes has been considered to be associated with oxidative stress. It has been suggested that increased free radicals and decline of antioxidant defense mechanisms induce diabetic micro- and macrovascular complications (1–3). Vitamin C is one of the major antioxidants and is detected in various blood components (4). However, measurements of vitamin C levels have shown inconsistent results, and the interpretation of vitamin C levels in diabetes as an antioxidant biomarker has not been clarified (5–8). In this study, we investigated the lymphocyte and plasma vitamin C levels in type 2 diabetic patients with and without diabetes complications.

RESEARCH DESIGN AND METHODS

— Forty-one patients with type 2 diabetes (63 ± 8.9 years [mean ± SD]; 25 men and 16 women) attending the Department of Endocrinology and Metabolism at Shizuoka City Hospital were recruited. Type 2 diabetes was diagnosed according to the American Diabetes Association criteria. The duration of illness was 11 ± 8.3 years, fasting plasma glucose was 137 ± 43 mg/dl, and HbA_{1c} levels were 7.1 ± 1.0%. Twenty-six patients had diabetes complications with neuropathy, retinopathy, or nephropathy, and 15 patients had no complications. Both diabetic groups were matched by age, sex, fasting plasma glucose, and HbA_{1c} level (63 ± 9.7 years, 18 men and 8 women, 137 ± 45 mg/dl, and 7.2 ± 1.0% for diabetic patients with complications compared with 64 ± 7.5 years, 7 men and 8 women, 137 ± 42 mg/dl, and 6.8 ± 0.8% for diabetic patients without complications, respectively). The duration of illness was longer in the diabetic patients with complications than in diabetic patients without complications (13 ± 9.1 vs. 7.7 ± 5.2 years, respectively, $P = 0.051$). For the normal control subjects, 50 age- and sex-matched healthy volunteers (63 ± 5.7 years, 31 men and 19 women) were recruited. The participants taking vitamin supplements were excluded from the study. All participants gave informed consent before entering the study. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee at the hospital.

Blood samples were obtained by vein puncture in the morning while the patients were in the fasting state. Lymphocytes and plasma were prepared by centrifugation and the Ficoll gradients method, then immediately treated with metaphosphoric acid (final 5% wt/wt) to stabilize vitamin C (9,10). These processes were performed within 2 h under cooled conditions on ice to obtain reliable data. The vitamin C samples were stored at −80°C until analyzed, and the vitamin C (ascorbic acid, reduced form) levels were measured by high-performance liquid chromatography with the electrochemical detector method (11). All samples were handled and stored similarly in both diabetic patients and control subjects.

The lymphocyte and plasma vitamin C levels in type 2 diabetic patients were compared with those of the control subjects. The differences between the vitamin C levels in type 2 diabetic patients with and without diabetes complications were also studied. Statistical analysis was performed with the unpaired Student's *t* test to compare the data between diabetic patients and control subjects and between type 2 diabetic patients with and without diabetes complications. A P value <0.05 was considered significant.

RESULTS

— The lymphocyte vitamin C level in diabetic patients was significantly lower than in control subjects (18 ± 4.5 vs. 28 ± 7.9 nmol/mg protein, $P < 0.0001$), whereas the plasma vitamin C level was not different (59 ± 19 vs. 53 ± 18 μmol/l, $P = 0.17$) (Fig. 1*A* and *B*). There were no significant linear correlations between the lymphocyte and plasma vitamin C levels in diabetic patients ($r = 0.011$, $P = 0.95$) as well as in control subjects ($r = 0.14$, $P = 0.35$). The lymphocyte vitamin C level in diabetic patients with complications was significantly lower than in those without complications (17 ± 3.3 vs. 21 ± 5.4 nmol/mg protein, $P = 0.011$) (Fig. 1*C*), whereas the plasma vitamin C level was not different (59 ± 18 vs. 59 ± 21 μmol/l, $P = 0.97$).

CONCLUSIONS

— Increased oxidative stress in diabetes could contribute to depletion of antioxidants such as vitamin C (2,3). In this report, we demonstrated that the lymphocyte vitamin C level is significantly lower in type 2 diabetic patients, but we could not observe such an association in plasma vitamin C levels. The plasma concentration of vitamin C is considered to be strongly correlated with transient consumption of foods such as fruit, supplements, and vegetables (4).

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

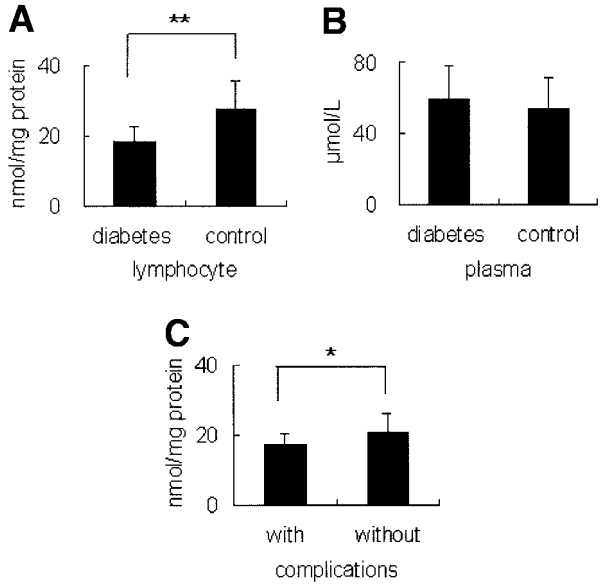

Figure 1—*Lymphocyte and plasma vitamin C levels in type 2 diabetic patients* (n = *41*) *and control subjects* (n = *50*). A*: Lymphocyte vitamin C level in diabetic patients was significantly lower than that in the control subjects* (**P < *0.0001*). B*: Plasma vitamin C level in diabetic patients was not different from that in the control subjects* (P = *0.17*). C*: Lymphocyte vitamin C level in diabetic patients with complications* (n = *26*) *was significantly lower than that in those without complications* (n = *15*) (*P = *0.011*). *The horizontal bars represent the mean ± SD.*

Compared with plasma, lymphocyte has been reported to maintain a vitamin C concentration as large as 80- to 100-fold across the plasma membrane (12,13) and to have cell-membrane transporting mechanisms between vitamin C and glucose (14,15). In diabetes, therefore, the measurement of lymphocyte vitamin C might be expected to be a more reliable antioxidant biomarker than plasma vitamin C level.

It is unclear whether leukocyte vitamin C correlates with diabetes complications. VanderJagt et al. (5) reported that vitamin C levels in mononuclear leukocytes were decreased in the whole group of type 1 diabetic patients compared with control subjects but were not different between patients with and without long-term complications. We showed the significant lower lymphocyte vitamin C levels in patients with type 2 diabetes with complications compared with those without complications. However, the results should be interpreted carefully because of the small sample size and because the differences of lymphocyte vitamin C level among different diabetes complications are not fully clarified. Further studies are required to investigate the precise correlations of lymphocyte vitamin C with duration or severity of diabetes and to establish the clinical usefulness of lymphocyte vitamin C level as a biomarker in developing diabetes complications.

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References

1. Giugliano D, Ceriello A, Paolisso G: Oxidative stress and diabetic vascular complications. *Diabetes Care* 19:257–267, 1996
2. Maritim AC, Sanders RA, Watkins JB 3rd: Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 17: 24–38, 2003
3. Hasanain B, Mooradian AD: Antioxidant vitamins and their influence in diabetes mellitus. *Curr Diab Rep* 2:448–456, 2002
4. Omaye ST, Schaus EE, Kutnink MA, Hawkes WC: Measurement of vitamin C in blood components by high-performance liquid chromatography: implication in assessing vitamin C status. *Ann N Y Acad Sci* 498:389–401, 1987
5. VanderJagt DJ, Harrison JM, Ratliff DM, Hunsaker LA, Vander Jagt DL: Oxidative stress indices in IDDM subjects with and without long-term diabetic complications. *Clin Biochem* 34:265–270, 2001
6. Cunningham JJ, Ellis SL, McVeigh KL, Levine RE, Calles-Escandon J: Reduced mononuclear leukocyte ascorbic acid content in adults with insulin-dependent diabetes mellitus consuming adequate dietary vitamin C. *Metabolism* 40:146–149, 1991
7. Sinclair AJ, Taylor PB, Lunec J, Girling AJ, Barnett AH: Low plasma ascorbate levels in patients with type 2 diabetes mellitus consuming adequate dietary vitamin C. *Diabet Med* 11:893–898, 1994
8. Schorah CJ, Bishop N, Wales JK, Hansbro PM, Habibzadeh N: Blood vitamin C concentrations in patients with diabetes mellitus. *Int J Vitam Nutr Res* 58:312–318, 1988
9. Margolis SA, Davis TP: Stabilization of ascorbic acid in human plasma, and its liquid-chromatographic measurement. *Clin Chem* 34:2217–2223, 1988
10. Umegaki K, Yoshimura M, Nishimuta M, Esashi T: A practical method for determination of vitamin C in plasma by high-performance liquid chromatography with an electrochemical detector. *J Jpn Soc Nutr Food Sci* 2:107–111, 1999
11. Washko PW, Hartzell WO, Levine M: Ascorbic acid analysis using high-performance liquid chromatography with coulometric electrochemical detection. *Anal Biochem* 181:276–282, 1989
12. Bergsten P, Amitai G, Kehrl J, Dhariwal KR, Klein HG, Levine M: Millimolar concentrations of ascorbic acid in purified human mononuclear leukocytes: depletion and reaccumulation. *J Biol Chem* 265: 2584–2587, 1990
13. Evans RM, Currie L, Campbell A: The distribution of ascorbic acid between various cellular components of blood, in normal individuals, and its relation to the plasma concentration. *Br J Nutr* 47:473–482, 1982
14. Bergsten P, Yu R, Kehrl J, Levine M: Ascorbic acid transport and distribution in human B lymphocytes. *Arch Biochem Biophys* 317:208–214, 1995
15. Ngkeekwong FC, Ng LL: Two distinct uptake mechanisms for ascorbate and dehydroascorbate in human lymphoblasts and their interaction with glucose. *Biochem J* 324:225–230, 1997