

Assessment of Antioxidant Vitamins Retinol and α -Tocopherol in Plasma and Ascorbic Acid in Plasma and Mononuclear Leukocytes in Type 2 Diabetics

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The incidence of Type 2 diabetes is on the rise and along with this increase there is a growing interest in the efficacy of antioxidant vitamin therapy in the treatment of this malady.

Materials and Methods: In this case-control study, the levels of antioxidant vitamins retinol, α -tocopherol and ascorbic acid were measured with HPLC and colorimetric method, in plasma and mononuclear leukocytes of patients with diagnosed Type 2 diabetes (n=62).

Results: We observed significantly lower ascorbic acid levels in mononuclear leukocytes in our study group when compared to controls.

Conclusion: While these results are not definitive, we believe they do indicate that further studies are needed to include a larger segment of the population and an evaluation of dietary intakes and habits.

Key words: Retinol, α -tocopherol, Ascorbic acid, Type 2 diabetes, Antioxidants

Received: 09/2/2006- Accepted: 07/05/2007

Introduction

The prevalence of clinically diagnosed Type 2 diabetes is expected to increase worldwide to more than 300 million by the

year 2025.¹ Life-style modification, obesity and changes in dietary habits and composition may predispose a person to this common form of diabetes.^{2,3} Much attention has been focused recently on the antioxidant micronutrients retinol (vitamin A), α -tocopherol (vitamin E) and ascorbic acid (vitamin C) for their beneficial and therapeutic effects. Even though lack of consistency remains in some results, evidence from several studies reveals that altered plasma status of these antioxidants may have a role in occurrence of oxidative insults in this chronic disease.⁴⁻⁷ Positive association has been documented between oxidative stress and etiology of diabetic complications.^{2,8} It has been postulated that an increase in the status of antioxidants by consuming fruits and vegetables rich in exogenous antioxidants can protect against oxidative stress associated with Type 2 diabetes and this would also reflect levels of intake during acute oxidative activity.⁹⁻¹¹

This study was carried out to assess the concentrations of essential vitamins retinol and α -tocopherol in plasma and water soluble vitamin C both in plasma and mononuclear leukocytes (MN) of Type 2 diabetic patients and healthy controls in small segment of our population.

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Materials and Methods

All eligible participants in this study signed informed consent forms and the protocol was approved by the University Ethics Committee. Patients with Type 2 diabetes ($n=62$) with a mean age of 51.30 ± 9.19 years were recruited from the diabetic center at the university hospital. Duration of disease was 9.99 ± 7.81 years. Patients with hyperlipidemia, those who smoked or who had any unusual dietary habits or systemic disease such as nephropathy were excluded. Control subjects ($n=38$) consisted of non-diabetic healthy individuals with a mean age of 50.82 ± 10.74 years and were chosen from the same socio-demographic group as the study population.

None of the participants were taking vitamin or mineral supplements shortly before or during the study. Seven patients controlled their diabetes with diet alone, 37 treated with oral hypoglycemic medicine and 18 with insulin. Sufficient fasting blood samples were obtained by venous puncture for all biochemical analysis. Plasma levels of vitamins A and E were measured by reversed phase high-performance liquid chromatography (HPLC) and expressed as $\mu\text{g/mL}$.¹² Ascorbic acid concentration in plasma and MN was measured by 2,4 dinitrophenyl hydrazine as described by Roe and Keuther and expressed as mg/dL and mg/g TP , respectively.¹³ Laboratory assay for MN preparation was according to Cunningham.¹⁴

Plasma glucose concentration was measured by glucose-oxidase technique; glycated Hemoglobin (HbA1c) was measured by calorimetric and total protein (TP) content of cell protein precipitate was determined by the biuret method in our laboratory. Statistical calculations were performed using SPSS-10 for Windows software package and all results are presented as mean $\pm$ SD. Comparison between diabetic and control subjects was made with unpaired Student's T-test. For other variables, analysis of variance ANOVA was used when necessary. P value of < 0.05 was considered as significant.

Results

The observation (mean or %), characteristics and biochemical measurements of all participant are summarized in Table 1. These results indicate, comparing the mean plasma concentration of retinol from diabetic patients ($0.59 \pm 0.17 \mu\text{g/mL}$) and control subjects ($0.51 \pm 0.13 \mu\text{g/mL}$), no statistically significant difference. Plasma concentration for α -tocopherol among patients ($19.57 \pm 6.8 \mu\text{g/mL}$) and control subjects ($20.08 \pm 7.39 \mu\text{g/mL}$) was also not statistically significant. MN ascorbic acid level in diabetic patients was statistically lower than that of control subjects (1.35 ± 0.55 vs. $1.77 \pm 0.61 \text{ mg/g TP}$, $P < 0.05$). However, the plasma ascorbic acid level between two groups we observed showed no significant differences (0.99 ± 0.28 vs. $1.00 \pm 0.23 \text{ mg/dL}$).

Discussion

Our data did not reveal any statistically significance when considering mean plasma concentrations of retinol, α -tocopherol and ascorbic acid among type 2 diabetic patients and our control volunteers. Similar results on plasma concentration of retinol and α -tocopherol was reported by Ahmad and Basualdo from Type 2 diabetic subjects.^{4,15} Merzouk showed ascorbic acid levels in plasma of Type 2 diabetics and non- diabetics to be normal.⁷ Kim demonstrated that there were no significant differences between plasma ascorbic acid levels in diabetic patients and control group.¹⁶ However, several authors have reported both a decrease or higher levels of retinol, α - tocopherol and ascorbic acid concentrations when comparing Type 2 diabetics to control groups.^{4,6,7,16,17}

Epidemiological studies have confirmed that increased serum α -tocopherol levels are associated with decreased risk of Type 2 diabetes.¹⁸ Pharmacological doses have been shown to improve glycemic control and insulin-mediated glucose disposal.¹⁹ However, there have been suggestions that dietary in-

Table 1. Baseline characteristics and biochemical measurement of Type 2 diabetic and control subjects

	Healthy controls N=38	Type 2 patients N=62
Age (y)	50.8± 10.7	51.3 ± 9.1
Gender (M/F)	24/14	33/29
Duration of Diabetics (year)	-----	9.99 ± 7.81
Fasting Plasma glucose(mg/dL)	89±74	200 ± 52*
HbA1c (%)	-----	6.67 ± 1.04
Blood Pressure(mmHg):		130 ± 21
Systolic	110±12	79.4 ± 11.6
Diastolic	71.0±4.3	
Retinol (µg/mL)	0.51±0.13	0.59 ± 0.17
α- Tocopherol (µg/mL)	20.08±7.39	19.57 ± 6.8
Ascorbic Acid:		
Plasma (mg/dL)	1.00±0.23	0.99 ± 0.28
MN (mg/g TP)	1.77±0.61	1.35 ± 0.55*

Data is presented as mean ± SD or %; Comparison between groups has been made using student t-test;

* P<0.05

take of α-tocopherol is beneficial and has a protective effect on glucose metabolism.⁹

There is convincing evidence that ascorbic acid metabolism is altered in conditions such as hyperglycemia and insulin insufficiency and patients with diabetes have about 30% lower plasma ascorbic acid levels than persons without.^{8,20} Decrease in active transport of reduced ascorbic acid and uptake inhibition of the oxidized form been observed in hyperglycemia.^{21,22}

Unlike plasma concentration of ascorbic acid in our patients, concentration of this water soluble vitamin in MN, comparing to our controls, was lower ($P < 0.05$). Other investigators have also shown MN ascorbic acid to be reduced. Chen reported a significant reduction in Type 2 diabetics relative to non-diabetics (1.44 mg/g TP vs. 2.44 mg/g TP)²³ Cunningham observed a 30% reduction in adults with Type 1 diabetes.¹⁴ Mononuclear leukocytes can accumulate a much higher concentration of ascorbic acid than plasma and is considered to be the best index and

more reliable index of tissue ascorbic acid stores.²⁴

Plasma concentration of antioxidant vitamins of our study population was normal when comparing to that of controls however, interpretation of these result must be taken with caution and further research on a larger scale is encouraged which should include dietary intake of the population under investigation.

Acknowledgements

We are indebted to the head and staff of the university research division and also to The Cellular Molecular Research Center for financial support and technical assistance. We also gratefully acknowledge Ehsan Noormohammadi, BSc (Department of Biochemistry, University of Oklahoma) for his skillful editing of the manuscript and S. Teemsar for typing. All authors contributed to the interpretation of results.

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