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NATURALLY OCCURRING ANTIOXIDANT VITAMIN LEVELS IN PATIENTS WITH TYPE-II DIABETES MELLITUS

Muhammad Ahmad, Mudassir Ahmad Khan*, Abdus Sattar Khan
Department of Chemistry, University of Peshawar and *Department of Biochemistry & Clinical Chemistry, Khyber Medical College, Peshawar, Pakistan

Background: Type II diabetes mellitus is characterized by an inability of peripheral tissues to respond to insulin and the dysfunction of the endocrine pancreas to compensate for this resistance. Diabetes mellitus is associated with increased lipid peroxidation. Increased levels of lipid peroxides have been implicated in the pathogenesis of diabetic complications. To control and reduce the deleterious effects of lipid peroxides exist several antioxidant protective mechanisms. They comprise of enzymatic defenses and naturally occurring vitamins. The study was designed to determine and compare the antioxidant vitamin levels in Type II diabetic individuals and control healthy subjects. **Methods:** Blood glucose, triglycerides, total cholesterol, HDL-c, LDL-c, VLDL-c and antioxidant vitamins (β-Carotene, A, E, and C) levels were determined in 36 Type II diabetic patients and 30 healthy subjects. **Results:** Values of blood glucose and lipid parameters were observed to be significantly increased ($P < 0.001$) with the exception of HDL-c, which was significantly decreased ($p<0.001$) in Type II diabetes mellitus patients. The levels of antioxidant vitamins (β-Carotene, E, and C) were found to be significantly low in Type II diabetes mellitus group whereas the concentration of vitamin A showed a non-significant change when both Type II and control healthy groups were compared. **Conclusions:** Low levels of antioxidant vitamins observed in the study suggest, that Type II diabetes mellitus patients have significant defects of antioxidant protection.

INTRODUCTION

Type II (non-insulin dependent) diabetes mellitus is characterized by an inability of peripheral tissues to respond to insulin (insulin resistance) and the dysfunction of the endocrine pancreas to compensate for this resistance (relative insulin deficiency)[1-4]. The earliest abnormality that appears in pre-diabetic people is insulin resistance, which initially, in order to maintain normoglycemia, is accompanied by sufficient hyperinsulinemia[5]. Resistance to insulin is genetically determined[6] and is a major risk factor for the development of Type II diabetes mellitus[7]. Obesity, aging[8], and physical inactivity are the other risk factors associated with insulin resistance[9].

The persistence of hyperglycemia in diabetic patients, leads to generation of oxygen free radicals through glucose auto-oxidation and non-enzymatic glycation[10]. All of the major class of biomolecules is attacked by the free radicals, but lipids probably are more susceptible. The oxidative destruction of lipids by the free radicals is known as “lipid peroxidation”[11]. Diabetes mellitus is associated with increased lipid peroxidation. Increased levels of lipid peroxides have been implicated in the pathogenesis of diabetic complications[12].

To control and reduce the deleterious effects of lipid peroxides exist several antioxidant protective mechanisms. They constitute a primary defensive system that includes enzymatic defenses (glutathione peroxidase and superoxide dismutase, which depends on the presence of ions such as selenium, zinc, copper, and manganese) and naturally occurring vitamins such as vitamin A (retinol), C (ascorbic acid) and E (a-tocopherol), and beta-carotene. Recently a

great interest in scientific and public communities has raised due to the possible role of antioxidant vitamins in particular vitamin E, C and beta-carotene, to prevent lipoprotein oxidation and to antagonize the atherosclerosis process[13].

Keeping all this in view, in the present study we determined the levels of both the water-soluble and lipid-soluble naturally occurring antioxidant vitamins in order to know the antioxidant vitamin status of the diabetic patients and the non-diabetic healthy individuals.

MATERIALS AND METHODS

Thirty-six previously diagnosed Type II diabetes mellitus patients and thirty healthy individuals having no history of diabetes mellitus, coronary heart disease, hypertension or any other disease participated in this study. All diabetic patients and control subjects taking lipid lowering drugs and those on multivitamins, especially antioxidant vitamins, were not included.

Blood samples were collected after an overnight fast of 12-14 hours from the Type II diabetics as well as the control subjects. Serum was separated from the blood and glucose determination was done immediately. Remaining serum was stored at -20 °C until analyzed for the other parameters. Serum glucose, triglycerides, cholesterol and HDL-c were determined by enzymatic colorimetric method, using kits supplied by BioSystems, Spain. LDL-c and VLDL-c were calculated by Friedewald's formula[14] and Wilson's formula[15] respectively. Serum retinol and b-carotene were determined by using the analytical method proposed by Bradley and Hornbeck[16], whereas serum a-tocopherol assay was done by method recommended by Baker and Frank[16]. Ascorbic acid in the sample reduces 2,6-dichlorophenol-indophenol, a dye, from blue color to colorless form. When excess dye is added to a solution containing ascorbic acid the decrease in color, determined in a spectrophotometer, is a measure of the amount of ascorbic acid present[17]. The results were expressed as mean±SD and all statistical comparisons were made by applying Student's t-test and $p<0.05$ was considered as a significant value between two groups.

RESULTS

Blood glucose and lipid profile of Type II diabetes mellitus patients (34-66 years) and control subjects (26-60 years) is shown in Table-I. Body mass index of Type II diabetic group (25.25±3.82 kg/m^2) was significantly higher ($p<0.001$) as compared to control group (22.97±1.67 kg/m^2). The levels of fasting serum glucose, triglycerides, total cholesterol, LDL-c and VLDL-c were found to be significantly elevated ($p<0.001$), whereas the level of HDL-c showed a significant decrease ($p<0.001$) in Type II diabetic patients as compared to the control subjects. The comparison of antioxidant vitamin status between control subjects and Type II diabetic patients was also done (Table-II).

Table-1: Blood glucose and lipid profile of control subjects and type-II diabetic individuals

	CONTROL (n=30)	**TYPE-II DIABETES MELLITUS (n=36)**
Male/female Ratio	19:11	18:18
Age (Years)	42.40± 9.89	43.05±7.31¶
Glucose(mg/dl)	91.87± 6.32	232.78±44.21*
Triglycerides(mg/dl)	131.70±13.29	191.72±3.70*

Cholesterol(mg/dl)	179.53±23.68	236.83±31.54*
HDL-c (mg/dl)	51.87±8.74	34.83±5.31*
LDL-c (mg/dl)	101.37±26.80	163.61±29.83*
VLDL-c (mg/dl)	26.33±2.73	38.69±6.95*

*p<0.001 as compared to Control subjects.
¶: p value non-significant as compared to Control subjects.

The levels of all the antioxidant vitamins were found to be significantly low in Type II diabetes mellitus group when compared with healthy control group. On the other hand a non-significant difference in the level of serum retinol was observed on comparison of the two groups of subjects.

Table-2: Antioxidant vitamin levels of control subjects and Type-II diabetic individuals

	CONTROL (n=30)	TYPE-II DIABETES MELLITUS (n=36)
b-Carotene (mg/l)	0.99±0.46	0.66±0.26*
Vitamin A [Retinol] (mg/l)	0.47±0.10	0.49±0.15¶
Vitamin E [a-Tocopherol] (mg/l)	11.73±2.40	9.33±1.37*
Vitamin C [Ascorbic Acid] (mg/dl)	10.10±1.90	7.19±1.86*

*p<0.001 as compared to Control subjects.
¶ p value non-significant as compared to Control subjects.

DISCUSSION

Type II diabetes mellitus is one of the major health problems globally that affects over 124 million individuals worldwide[18]. It affects at least 5% of the population in the industrialized world[19]. Type II diabetes mellitus is about 8 to 10 times more common than Type I (insulin-dependent) diabetes mellitus[20]. Major disturbances in lipoprotein metabolism in Type II diabetic individuals are reflected by an increase in plasma triglycerides and low HDL-c, with normal or near normal LDL-c levels[21]. Okubo and Murase[22] reported hypertriglyceridemia and low high-density lipoprotein cholesterol levels in Japanese patients with Type II diabetes mellitus. They also stated that this combined abnormality was more common in patients with poor glycemic control. U.K. Prospective Diabetes Study 27[23] showed higher levels of mean total cholesterol and low-density lipoprotein cholesterol levels in Type II diabetes mellitus at diagnosis. Agboola-Abu and his group[24] noticed raised triglycerides, total cholesterol, low-density lipoprotein cholesterol and lower high-density lipoprotein cholesterol in newly diagnosed Type II diabetes mellitus patients. Our results also show like the aforementioned workers, elevated levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol and decreased levels of high-density lipoprotein cholesterol in Type II diabetic patients when compared to control subjects.

Patients with diabetes mellitus develop atherosclerotic vascular disease earlier and with greater severity than non-diabetic subjects[25]. Free oxygen radicals are one of the factors, which participate via lipid peroxides in the development of atherosclerosis[26]. Increased lipid peroxidation in diabetes mellitus is due to an altered intracellular ratio between free radicals and antioxidant systems[27].The imbalance between free radical production and antioxidant capacity leads to oxidative stress, which in turn is associated with the development of cardiovascular disease[28].

Many independent studies[29-31] reported significantly lower levels of serum b-carotene in patients with Type II diabetes mellitus than control group. Abahusain and his group[30] and Basualdo and colleagues[32] found similar mean serum retinol levels in Type II diabetes mellitus patients and the healthy control group. Polidori et al[31], on the other hand reported significantly lower vitamin A levels in Type II diabetes mellitus patients as compared to the control subjects. The results of our study show a non-significant change in retinol and significantly decreased b-carotene levels in Type II diabetes mellitus group as compared to control subjects.

Vitamin E is the major lipid-soluble vitamin. It is responsible for protecting the polyunsaturated fatty acids in membranes against lipid peroxidation. It protects lipids by scavenging peroxyl radicals by breaking chain propagation steps[33]. Serum alpha-tocopherol levels in our study were significantly decreased in Type II diabetics as compared to control individuals. Gribauskas et al[34] found significantly lower levels of serum a-tocopherol in male patients with diabetes mellitus than normal control healthy subjects. Tavridou and his group[35] found no difference in a-tocopherol concentration in Type II diabetes mellitus patients as compared to control subjects. Reunanen et al[29] and Polidori et al[31] reported lower serum a-tocopherol levels in patients with Type II diabetes mellitus than healthy control subjects.

A number of independent research groups[36-38] reported significantly decreased ascorbic acid concentrations in both Type I and Type II diabetic patients when compared to healthy control subjects. Will et al[39] reported significantly lower ascorbic acid levels in persons with newly diagnosed diabetes mellitus than those without diabetes. We also found significantly decreased ascorbic acid levels in Type II diabetic patients as compared to control subjects.

The clinical importance of the determination of antioxidant vitamins in Type II diabetes mellitus patients is to find out the antioxidant status because of the implication of oxidative damage by free radicals in the pathogenesis of vascular disease. These results show that patients with Type II diabetes mellitus have significant defects of antioxidant protection, which may increase vulnerability to oxidative damage and the development of diabetic complications. Further studies on complicated and uncomplicated Type II diabetic patients are required to elucidate the antioxidant status of these compounds and their role in Type II diabetes mellitus.

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Address for Correspondence

Dr. Abdus Sattar Khan, Department of Chemistry, University of Peshawar, Peshawar.