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Antioxidant Activity in Patients with Type II Diabetes

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SUMMARY: Oxidative stress has been shown to be involved in many diseases including Diabetes. The aim of this study was the determination of antioxidant capacity in plasma of diabetic patients. The degree antioxidant capacity was calculated with DPPH assay, a method based on the scavenging of the 2,2-diphenyl-1picrylhydrazyl (DPPH) free radical. Antioxidant activity was not statistically significantly lower than in control group (p<0.05).

INTRODUCTION

Free radicals are atoms or molecules that have one or more unpaired electrons in their outer layer. These atoms or molecules are unstable and try to react with other atoms or molecules near them. In short, oxidative stress occurs when an oxygen molecule splits into individual atoms with unpaired electrons, called free radicals. Free radicals, in normal quantities, protect the humans from viruses, parasites and germs. Besides, oxidative stress is necessary for the cell because it acts as a stimulus for the cell to grow, move, or multiply (1,3,4).

However, in large quantities, free radicals can oxidize and damage fats, proteins and DNA (9,10). It is probable to play an important role in neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's. Oxidative stress is associated with cardiovascular disease, as the oxidation of low-density lipoprotein (LDL) in the vascular endothelium is a precursor to the formation of atherosclerotic plaques. Oxidative stress has been shown to be involved in many other diseases, such as sickle cell disease, myocardial infarction, schizophrenia, bipolar disorder, and fragile X chromosome syndrome. Finally, oxidative stress seems to be behind the syndrome of chronic fatigue. Antioxidants substances are the body's natural defense against oxidation. Antioxidants are produced in our body but we can also get them from food. Examples of antioxidants produced in the body are glutathione, lipoic acid, coenzyme Q10, Vitamin C, Vitamin E, carotenoids, Polyphenols (13).

Diabetes mellitus (SD) is a disease characterized by an increase in blood sugar (glucose), which is mainly due to insufficient production of insulin. There are two main types of diabetes: Type 1 diabetes usually occurs in young people (although it can occur at any age). It is characterized by destruction of the pancreatic β - cells that produce insulin, making it necessary to inject insulin in order for the person to survive. Type 2 diabetes mainly affects older people, usually obese and there is often an inherited predisposition to its occurrence. The most common effect of the disease, hyperglycemia, leads over time to serious damage to various body systems, such as nerves and blood vessels (5,7,11).

Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. Antioxidant capacity in human plasma can be determined with spectrophotometric methods (2,6). DPPH is a method based on the scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Fig. 1) (6,8).

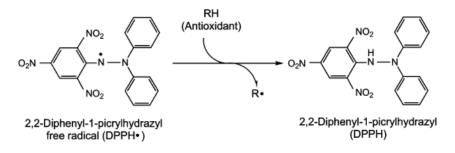


Figure 1: DPPH chemical reaction.

AIM

The objective of this study was the measurement of percentage decrease of DPPH in plasma of diabetic patients.

MATERIALS AND METHODS

A total of 40 venous blood specimens from 20 diabetes patients ($65,5 \pm 13$ age) and 20 healthy volunteers as controls ($55,5 \pm 18$ age) in Athens were collected in an EDTA containing vacutainer tube. Oxidative stress and antioxidant capacity was measured with DPPH assay.

EXPERIMENTAL PROCEDURE

Weigh 0.0020 g DPPH (MW: 394.32 g/mol) and dissolve in 25 mL CH $_3$ OH. Transfer the solution

to a 50.0 mL volumetric flask and make up to 50.0 mL with CH₃OH. The solution is stored in small volume polypropylene vials at -40° C.

25 µL of samples were mixed with 475 µL of 10 mM phosphate buffered saline (PBS), pH 7.4. 500 µL of 0.1 mM DPPH solution in methanol was added. The mixture was kept for 30 min in darkness temperature, before at room absorbance reading at 520 nm against blank, using a spectrophotometer (SmartSpec 3000 UV/Vis, Bio-Rad). The absorbance of the sample was compared with that of a reference sample containing only PBS and DPPH solution. The percentage decrease of DPPH was calculated by applying the following equation:

% of inhibition = $[1 - (As/A0)] \times 100$,

where As is the absorbance of sample and A0 is the absorbance of the DPPH solution.

RESULTS

All diabetics had plasma glucose levels of 124-304 mg/dL. Plasma percentage decrease of DPPH was $39,4 \pm 13,2$ in control group and $44,3 \pm 11,7$ in group of diabetic patients. These differences were not statistically significant (Fig.2).

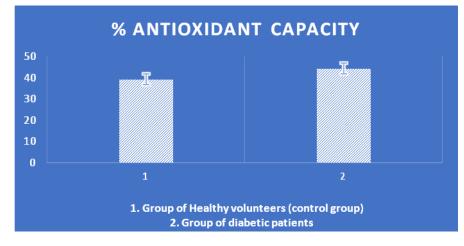


Figure 2: Plasma percentage decrease of DPPH.

CONCLUSIONS

Oxidative stress is the excessive concentration of free radicals in cells, which cause damage to cell function. The development of oxidative stress depends on the rate of production of free radicals within the cells, the rate of clearance of free radicals and the rate of repair of the damage it has caused (12). Diabetes is a dangerous disease where the body does not produce or use insulin properly (insulin resistance). This causes hyperglycemia and increased production of free radicals, causing oxidative stress. In this study there were not found significant differences between diabetic and non-diabetic subjects in terms of DPPH. Our findings suggest that the total antioxidant capacity may reduce the risk of complications in type 2 diabetes. More studies are necessary to understand the above biological mechanisms.

Conflicts of Interest: The authors declare no conflicts of interest.

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