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Free radical capacity in plasma of type 2 diabetic patients

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ABSTRACT

Type 2 diabetes is most common in middle age. This type of diabetes usually causes defects in insulin secretion. The disease worldwide is increasing day by day. The creation of free radicals that can occur in patients with diabetes on protein oxidation, lipid peroxidation; DNA can affect and damage the cells. The kidneys, eyes, cardiovascular system, blood vessels are so detrimental. This study aims to evaluate free radical capacity in plasma of type 2 diabetic Patients. This case - control study was conducted using random sampling. A survey was carried out on blood from samples, which included 30 type 2 diabetic patients, and 30-control sample of age and sex matched subjects. The free radical capacity level were significantly ($P = 0.005$) higher in control sample compared with type 2 diabetic patients. Free radicals have an important part in reducing cellular damage.

Key words: Radical capacity, type 2 diabetes, plasma

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1. INTRODUCTION

The purpose of this study was to evaluate plasma levels of free radicals in the population of type 2 diabetic patients compared with control subjects. Diabetes mellitus is a chronic metabolic disease and currently more than 347 million people worldwide are infected with this disease (1). The cause of diabetes or to diminish insulin due to destruction of beta cells (type I diabetes) or decreased responsiveness of peripheral insulin receptors is due to insulin resistance (type II diabetes). Diabetes symptoms include Hyperglycemia (high blood sugar), polyuria, water load, and the binge eating is characterized by the appearance of glucose in the urine (2,3). This syndrome for a long period is serious and irreversible. For example, retinopathy, nephropathy, neuropathy and vascular damage are associated (4,5). The production process (ROS), accumulation of reactive oxygen radicals, oxidative stress in tissues, particularly the pancreatic beta cells, causes chronic hyperglycemia of diabetes (6,7). The formation of these radicals causes damage to critical macromolecules, cells, membrane lipid peroxidation and finally damages cell (8,9). Free radicals,

including reactive oxygen species (ROS), nitrogen species (10). Antioxidant compounds in healthy subjects which deal With free radicals (11). Oxidative stress in type 2 diabetes increases the free radicals (12). The imbalance between the production of free radicals and radical scavenging capacity of antioxidants is called oxidative stress (13). Diabetes is associated with oxidative stress-induced ROS production, which can potentially harm the DNA insert (14). Moreover, it can damage the kidneys, cataracts, cardiovascular system - blood vessels and so forth. Oxidative stress results from an imbalance between ROS production and consumption. The production of free radicals is exacerbated by the presence of metals. In the case of oxidative stress, many are injured of macromolecules, process of lipid peroxidation, protein oxidation, oxidation Dena, activation of enzymes and stimulates membrane dysfunction (15,16). Available evidence indicates that oxidative stress is involved Patvzhnr more than a hundred diseases, including diabetes mellitus (17). In this context, several works have been proposed to increase the production of free radicals because of auto-oxidation of glucose and the formation of

glycated proteins. In addition, reduced activity or capacity of antioxidants can also be effective in enhancing the state (18). Diabetes is associated with OH at C1 to C2 in the presence of superoxide which leads to hydrogen peroxide oxidizes and causes DNA damage etc(19). One of the most aggressive radicals is hydroxyl radical (20), oxidative stress in diabetes and diabetic complications now as a mechanism is proposed (21). In this study, we conclude that oxidative stress plays a major role in diabetic patients and strengthen the antioxidant system; and we can prevent some of the damages.

Table1) and free radical capacity. Biochemistry the Auto analyzer Model BT-3000, barking exams and Pars Azmon kit. Measurement of plasma free radicals: a 20 µm patient serum, phosphate buffer 380 µm with PH=7.4 and 400 µm 1, diphenyl-2-picrylhydrazyl (DPPH) mixture, incubated for 30 min at room temperature and the absorbance read at a wave length of 520nm. In addition, samples with the spectrophotometer Model EPOCH-Bio Tek readings. T-

2. MATERIALS AND METHODS

This case - control study, and samples were taken randomly and were matched with age and sex. Patients with type 2 diabetes were considered in this research. Heparinized blood samples were obtained from patients. Prepared plasma was isolated from blood samples with the help of red blood cells by centrifuging (3000 RMP min to 10 min). The isolated plasma was used for biochemical tests (FBS, TG, CHO, HDL-T, LDL _T and A1C) (Test was used to analyze the data. Values of P <0.05 was considered significant.

Blanch methanol was used in this test.

Dpph rate is obtained from the following formula: %

$$DPPH = (A-AX)/A * 100 \%Inhibition$$

A = DPPH absorption along with methanol

AX = DPPH absorption along with plasma

Table1. Clinical characteristics of studied subjects

P.Val	Mean±SD	Mean±SD	Type for measurement
	Control case	Diabetic	
0.000	8.335±93.100	51.746±219.733	F.B.S
0.000	18.172±193.600	30.049±269.200	Cho
0.000	33.609±203.533	87.118±288.466	T.G
0.000	0.628±6.860	1.156±8.906	A1C
0.188	4.141±50.466	4.107±51.766	Ag
0.001	1.201±28.330	1.113±27.046	B.M.I
0.003	6.441±37.560	3.518±33.033	HDL - C
0.000	5.343±130.000	12.976±146.866	LDL - C

Results are expressed as mean ± SD. Statistical significance was achieved, as P values were less than 0.05. All statistical analysis was performed using the SPSS (version 18) independent – samples T-Test.

3. RESULTS AND DISCUSSION

We observed a significant (P=0.005) decrease level of free radical capacity 1, diphenyl-2-picrylhydrazyl (DPPH) method in type 2 diabetic patients in comparison to Control samples (0.942 ± 0.164 vs. 1.133 ± 0.276) (Chart 1) (Table2). The outcome of this study suggests that oxidative stress occurs in patients with type 2 diabetes as increased levels of blood glucose and related metabolic differences. Hyprglaysmy antioxidant system occurs in the body by increasing the production of free radicals (18). Several studies have been done based on this knowledge. Plasma total antioxidant power level was decreased in diabetes (22-24). Oxidative stress can accelerate clinical complications in type 2 diabetes (25-27). ROS damage by stimulating the beta cells to produce insulin can cause diabetes (28). Glucose concentration increases with the Russians in vivo as well as in cell cultures are associated

(29,30). Sheikh-Ali studies diabetic patients by increasing free radicals and ROS can damage the cell (31). Lipins K studies in diabetes foundation continuous glucose increased free radicals are produced (32). Due to the presence of oxidative stress in diabetic patients and further production of free radicals, lipid levels also increase the need for increase in antioxidants. Varieties of mechanisms of oxidative stress in diabetes mellitus have been reported in the press. Moreover, it is a sugar oxidation. Building components with alpha - hydroxy aldehydes (such as glucose) can become Enol form, with the revival of intermediate elements and oxygen free radicals. The first is the creation of radical. Superoxide radical and superoxide dismutase enzymes produce H₂O₂. It is the active species in the presence of transition metals to produce hydroxyl radicals, which can be extremely dangerous and aggressive (33). When blood glucose increases the sugar binding proteins, compounds are produced that are involved in the production of free radical Superoxide (34). radicals produced during the reaction shown in addition to the decrease in GSH levels, resulting in the activation of the NADPH production disturbance occurring poly-ol, a reconstruction of vitamins E and C, resulting in an

effective decrease in antioxidant capacity (35). It seems that in diabetes, oxidative stress causes due to the over production of free radicals and decreased levels of

antioxidants during oxidative stress.

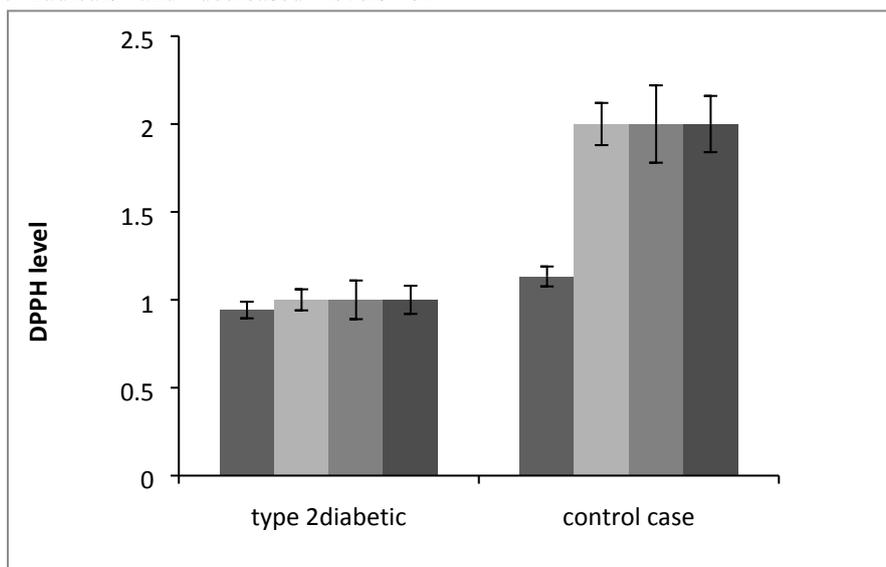


Chart 1: DPPH level in type 2 diabetic and control case

*P<0.05. The value is mean±SD.

Table2. Free radical capacity method DPPH

<i>P.val</i>	<i>Control case</i> <i>mean±SD</i>	<i>Type 2diabetic</i> <i>mean±SD</i>	
0.005	1.133 ± 0.276	0.942 ± 0.164	DPPH

4. CONCLUSION

Our study showed that there is a change of free radicals in type 2 diabetic patients. These changes can create irreparable damage in the lipid peroxidation, protein oxidation DNA and so on. In this state of diabetic patients, to minimize the harm vitamins E, C and antioxidants are beneficial.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

REFERENCES

1. Danaei G FM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since: 1980 systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2,7 million participants. *Lancet* 2011;378(1):31-40.
2. Poitout V. Glucolipotoxicity of the pancreatic beta-cell: myth or reality?

3. Yoon JW, Jun HS. Cellular and molecular roles of beta cell autoantigens, macrophages and T cells in the pathogenesis of autoimmune diabetes. *Archives of pharmacol research*. 1999;22(5):437-47.
4. Kahn BB FJ. Obesity and insulin resistance. *J Clin Invest*. 2000;106(4):473-81.
5. Luitse MJ, Biessels GJ, Rutten GE, Kappelle LJ. Diabetes, hyperglycaemia, and acute ischaemic stroke. *Lancet neurology*. 2012;11(3):261-71.
6. Jacob MH, Pontes MR, Araujo AS, Barp J, Irigoyen MC, Llesuy SF, et al. Aortic-banding induces myocardial oxidative stress and changes in concentration and activity of antioxidants in male Wistar rats. *Life sciences*. 2006;79(23):2187-93.
7. Tsutsui H, Kinugawa S, Matsushima S. Mitochondrial oxidative stress and dysfunction in myocardial remodelling. *Cardiovascular research*. 2009;81(3):449-56.
8. Scandalios JG. The rise of ROS. *Trends in biochemical sciences*. 2002;27(9):483-6.
9. Sindhu RK, Roberts CK, Ehdaie A, Zhan CD, Vaziri ND. Effects of aortic coarctation on aortic antioxidant enzymes and NADPH oxidase protein expression. *Life sciences*. 2005;76(8):945-53.
10. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes care*. 2004;27(5):1047-53.
11. Tesfamariam B. Free radicals in diabetic endothelial cell dysfunction. *Free radical biology & medicine*. 1994;16(3):383-91.
12. DJ B. What is oxidative stress. *Metabolism*. 2000;49(2):3-8.
13. Granot E KR. Oxidative stress in childhood—in health and disease states *Clin Nutr*. 2004;23(1):3-11.
14. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2003;17(10):1195-214.
15. Predy VR RM, Mantlc O, Peters TJ. Oxidative damage in liver diseases. *Journal of the International Federation of Clinical Chemistry*. 1998;10(1):16-9.
16. Harman D. Aging and oxidative stress. *Journal of the International Federation of Clinical Chemistry / IFCC*. 1998;10(1):24-7.
17. Pincemail J. Free radicals and antioxidants in human diseases. In: Favier AE CJ, Kalyanaraman B, Fontecave M, Pierre JL, editors. *Analysis of free radicals in biological system*. Basal: Birkhauser Verlag; 1995. p. 83-98.
18. Ceriello A. Acute hyperglycaemia and oxidative stress generation. *Biochemical Society transactions*. 2008;36(Pt 5):901-4.

- Diabetic medicine : a journal of the British Diabetic Association. 1997;14 Suppl 3:S45-9.
19. Gunther MR, Hanna PM, Mason RP, Cohen MS. Hydroxyl radical formation from cuprous ion and hydrogen peroxide: a spin-trapping study. *Archives of biochemistry and biophysics*. 1995;316(1):515-22.
 20. Burk RF, Ludden TM. Exhaled alkanes as indices of in vivo lipid peroxidation. *Biochemical pharmacology*. 1989;38(7):1029-32.
 21. SA M. Oxidative stress in diabetes mellitus. *Romaniaj Biophys*. 2008;13(3):225-36.
 22. Karasu C. Glycooxidative stress and cardiovascular complications in experimentally-induced diabetes: effects of antioxidant treatment. *The open cardiovascular medicine journal*. 2010;4:240-56.
 23. Ceriello A, Bortolotti N, Motz E, Crescentini A, Lizzio S, Russo A, et al. Meal-generated oxidative stress in type 2 diabetic patients. *Diabetes care*. 1998;21(9):1529-33.
 24. Vijayalingam S, Parthiban A, Shanmugasundaram KR, Mohan V. Abnormal antioxidant status in impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabetic medicine : a journal of the British Diabetic Association*. 1996;13(8):715-9.
 25. Haffner SM, Agil A, Mykkanen L, Stern MP, Jialal I. Plasma oxidizability in subjects with normal glucose tolerance, impaired glucose tolerance, and NIDDM. *Diabetes care*. 1995;18(5):646-53.
 26. Oberley LW. Free radicals and diabetes. *Free radical biology & medicine*. 1988;5(2):113-24.
 27. Nourooz-Zadeh J RA, Tajaddini-Sarmadi J, Tritschler H, Rosen P, Halliwell B, Betteridge DJ. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. *Diabetologia*. 1997;40(6):647-53.
 28. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes/metabolism research and reviews*. 2001;17(3):189-212.
 29. Coudray C, Roussel AM, Arnaud J, Favier A. Selenium and antioxidant vitamin and lipidperoxidation levels in preaging French population. EVA Study Group. *Edude de vieillissement arteriel. Biological trace element research*. 1997;57(2):183-90.
 30. Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochemical and biophysical research communications*. 1990;173(3):932-9.
 31. Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods in enzymology*. 1984;105:114-21.
 32. Halliwell B, Kaur H, Ingelman-Sundberg M. Hydroxylation of salicylate as an assay for hydroxyl radicals: a cautionary note. *Free radical biology & medicine*. 1991;10(6):439-41.
 33. Bhandari U, Kanojia R, Pillai KK. Effect of ethanolic extract of Zingiber officinale on dyslipidaemia in diabetic rats. *Journal of ethnopharmacology*. 2005;97(2):227-30.
 34. Mooradian AD. Antioxidants and diabetes. *Nestle Nutrition workshop series Clinical & performance programme*. 2006;11:107-22; discussion 22-5.
 35. Lipinski B. Pathophysiology of oxidative stress in diabetes mellitus. *Journal of diabetes and its complications*. 2001;15(4):203-10.