

Study of oxidative stress in type 2 diabetic patients and its relationship with glycated hemoglobin

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ABSTRACT

الأهداف: يزيد فرط سكر الدم أنواع الجذيرات الخالية والأكسجين المتفاعل التي تسبب التلف المتأكسد، والذي بدوره يؤدي إلى مضاعفات السكري طويلة المدى. وتهدف هذه الدراسة إلى تقييم نشاط الأنزيمات المضادة للأكسدة في مرضى السكري وكذلك من أجل تحديد العلاقة بين فرط السكر في الدم وفوق التأكسد للدهون.

الطريقة: شارك في هذه الدراسة 30 مريضاً يعانون من داء السكري من النوع الثاني و30 فرداً من الأصحاء يمثلون (مجموعة التحكم). تم تحويل المرضى إلى مستشفى سيناء (مدينة حمدان بإيران) في الفترة ما بين أبريل إلى يونيو 2006م. تم قياس هيموجلوبين أنزيم السكري (HbA1c) كمؤشر لزيادة سكر الدم باستعمال طريقة الإستشراب (النظام الحيوي) وتم تحديد مالون ديلدهيد (MDA) باستعمال طريقة مقياس اللون. تم تقييم نشاط فوق التأكسد للجولثاتون (GPx) والتأكسد الزائد للديسموتاس (SOD) باستعمال طريقة (UV-Vis) تقنية قياس الضوء الطيفي (طاقم راندوكس).

النتائج: كان متوسط (HbA1c) مرتفعاً لدى مرضى السكري مقارنة مع مجموعة التحكم الأصحاء، وكان الاختلاف ملحوظاً إحصائياً ($p < 0.001$). كان مصل (MDA) لدى مرضى السكري أعلى مقارنة مع مجموعة التحكم الأصحاء ($p < 0.001$). كان هنالك اختلافات ملحوظة في أنشطة (SOD) و(GPx) بين مجموعتي الدراسة، ويشير ذلك إلى النشاط المنخفض لدى مجموعة مرضى السكري ($p < 0.001$). كان هنالك علاقة ملحوظة بين (MDA) و(HbA1c) في مجموعة مرضى السكري والأصحاء.

خاتمة: أظهرت البيانات زيادة في الدهون فوق التأكسد وضغط التأكسد في مرضى السكري وتشير أيضاً إلى العلاقة الإيجابية بين درجة فرط السكر في الدم وضغط التأكسد. وقد يساعد تقييم حالة التأكسد واختيار المعالجة الملائمة في مساندة دعم مضادات الأكسدة لدى هؤلاء المرضى.

Objectives: To evaluate the activity of antioxidant enzymes in diabetic patients and also to determine the correlation between hyperglycemia and lipid peroxidation.

Methods: Thirty patients with type 2 diabetes and 30 healthy individuals (control group) participated in this case-control study. The patients were referred to Sina Hospital, Hamadan, Iran from April to June 2006. Glycated hemoglobin (HbA1c) was measured as a marker of hyperglycemia using the chromatography method (Biosystem) and malondialdehyde (MDA) was determined using the colorimetric method. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity were assessed using the UV-Vis spectrophotometric technique (Randox kit).

Results: The mean of HbA1c was higher in diabetic patients compared to the healthy group, and the difference was statistically significant ($p < 0.001$). Serum MDA in diabetics was higher compared to those of healthy subjects ($p < 0.001$). There were significant differences in activities of SOD and GPx between the 2 studied groups indicating lower activity in diabetic patients ($p < 0.001$). There was a significant relationship between MDA and HbA1c in diabetic and healthy subjects.

Conclusion: The data showed an increase in lipid peroxidation and oxidative stress in diabetes and also indicated a positive correlation between the degree of hyperglycemia and oxidative stress. Evaluation of oxidative status and choosing the appropriate treatment may help to support antioxidant defense in these patients.

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One of the major hypotheses proposed to explain the hyperglycemia-induced onset of diabetic complications is an increase in oxidative stress.¹ Hyperglycemia can stimulate reactive oxygen species (ROS) production from a variety of sources. Production of ROS depletes antioxidants and antioxidant enzymes, leading to additional ROS accumulation. Plasma measure of oxidative stress is increased in patients with diabetes compared to control subjects.² Protein glycation is important in leading to diabetic microvascular complications. Protein glycation is widespread, and glycation of hemoglobin (HbA1c) probably reflects the level of glycation of other proteins.^{3,4} This test provides an index of the average blood glucose concentration over the life of the hemoglobin molecule (approximately 6 weeks). Advanced glycation end product (AGE) formation is dependent on oxidative processes and can create ROS through the Millard reaction. Markers include the enzymatic activities of catalase, superoxide dismutase (SOD), glutathione peroxidases (GPx), and glutathione reductase (GSH-Rx), as well as thiobarbituric acid reactive substance (TBARS) levels, an indirect measurement of free radical production has been shown consistently elevated in diabetes.⁵ In an animal study, vitamins C and E were shown to decrease TBARS, GPx activity while increasing catalase and SOD activities when compared to unsupplemented diabetic animals.⁶ The SOD activity is undoubtedly important to the regulation of oxidative stress in diabetes. However, there is variation in the status of this enzyme in the diabetic state. Some studies have reported decreased SOD activity,^{6,7} while others have shown increase⁸ or no change in the enzyme.^{9,10} Turk et al¹¹ showed an increase in SOD activity and decrease in catalase activity and suggested that these alterations may be owing to the compensatory mechanisms of the antioxidant system in type 2 diabetes.¹¹ Excessive production of free radicals were observed both in type 1 and type 2 diabetes.¹² Griesmacher et al¹³ showed increased lipid peroxidation leading to elevated free radicals in both type 1 and 2 diabetes. Mahboob et al,¹⁴ studying lipid peroxidation, and antioxidant enzyme levels in male and female diabetic patients indicated that an increase in lipid peroxidation product MDA and decline in glutathione dependent antioxidant defense may appear early in type 2 diabetes patients. There is also evidence showing erythrocyte GPx activity, glutathione content and plasma beta-carotene to be significantly lower in diabetic patients compared to control subjects, but with no significant difference between patients with or without subclinical complications.¹⁵ In these studies, there is no evidence showing the relationship of hyperglycemia and lipid peroxidation or antioxidant enzyme activities. The aim of our research was to measure antioxidant enzyme

activity and lipid peroxidation markers in patients with type 2 diabetes. Furthermore, we analyzed the possible correlation between lipid peroxidation and glycemic control levels in diabetic patients.

Methods. The present study was conducted on 30 patients (14 females, 16 males) with type 2 diabetes who attended the Educational and Therapeutic Center, Sina Hospital, Hamadan, Iran for their routine medical examination, and 30 healthy age matched controls (15 females, 15 males). All the patients were taking oral anti-diabetic drugs at the time of study. All studied subjects were non-smokers and normotensive. The patients did not have any other complications of diabetes. Informed consent was obtained from all subjects, and the study project was reviewed and approved by the ethics committee of Islamic Azad University, Tehran, Iran.

Glutathione peroxidase activity measurement. Determination of GPx activity was performed using a kit (RANSEL, RANDOX Lab, Crumlin, Co Antrim, UK) based on the Paglia and Valentine method.¹⁶ In this method, GPx catalyses the oxidation of glutathione (GSH) by Cumene hydroperoxide.¹⁶ In the presence of glutathione reductase, and nicotinamide adenine dinucleotide phosphate (NADPH) the oxidized GSH (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺, and consequently the decrease in absorbance at 340 nm is measured.¹⁶ The manufacturers have recommended to use Drabkin's reagent for dilution. This is due to the presence of peroxidases in human blood, which may give falsely elevated results and the addition of cyanide inhibits this interference. The GPx activity in whole blood was measured as unit per liter of hemolysate according to test procedure enclosed in the kit.

Superoxide dismutase activity measurement. To determine erythrocyte SOD activity we used the kit labeled RANSOD (RANDOX Lab, Crumlin, Co Antrim, UK) based on the method developed by McCord and Fridovich.¹⁷ The method of the kit employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction.

Measurement of biochemical parameters. Serum glucose, triglycerides, total cholesterol, and HDL-cholesterol levels were assayed using Randox kit (Randox Laboratories Ltd., UK).

Plasma malondialdehyde measurement. We used the modified colorimetric method to measure MDA concentration in plasma. This method was based on MDA reaction with thiobarbituric acid (TBA) and

resulting MDA-TBA2 complex was quantified by photometric reading at 535nm.¹⁸

Glycated hemoglobin measurement. Blood HbA1c level was measured by ion-exchange chromatography kit (Biosystem Company, Spain). In this method, after preparing the hemolysate where the labile fraction is eliminated, hemoglobins were retained by a cationic exchange resin. The HbA1c was specifically eluted after washing away the hemoglobin A1a+b fraction, and was quantified by direct photometric reading at 415nm.

Measurement of total hemoglobin. To determine hemoglobin concentrations in hemolysate prepared for antioxidant enzyme activity assay we used Hemoglobin Reagent Set Kit (Pointe Scientific Inc, Canton, USA) based on the cyanomethemoglobin method.

Statistics. Differences in measured parameters between normal and diabetic subjects were assessed using t-test. The relationships between parameters were analyzed using linear regression analysis. A probability of 0.05 was set as the level of statistical significance.

Results. Table 1 shows the clinical and biochemical data of the patient and control groups. Patients with type 2 diabetes had higher levels of glucose, total cholesterol, and triglycerides, but lower level of HDL-cholesterol. The differences of these data between patients and

control group were statistically significant ($p < 0.05$). Our main parameters of oxidative stress markers are shown in Table 2. Both GPx and SOD as indicators of antioxidant defense system had significantly lower activity in diabetic patients ($p < 0.001$). Plasma MDA and HbA1c were significantly higher in patients with type 2 diabetes compared to controls ($p < 0.001$). Correlation analysis showed a significant positive correlation between HbA1c and MDA in diabetic ($r = 0.38$, $p < 0.05$) and healthy groups ($r = 0.29$, $p < 0.05$). In diabetic patients, plasma MDA levels positively correlated with triglycerides ($r = 0.59$, $p < 0.05$).

Discussion. According to Diabetes Control and Complications Trial Research, hyperglycemia seems to be the main factor in developing diabetic microvascular complications.¹⁹ One of the major hypotheses proposed to explain the hyperglycemia induced onset of diabetic complications is an increase in oxidative stress.²⁰ Diabetes mellitus not only stimulates the generation of ROS, but also impairs the ability of a cell or tissue to cope with the increased oxidative burden.¹ The level of these ROS are controlled by the antioxidant defense system. The scope of our study is limited to the most extensively investigated enzymes, namely, SOD and GPx. Malondialdehyde, as a late stage lipid oxidation byproduct, accumulates in many disease states such as diabetes mellitus. Earlier studies reported increased levels of lipid peroxides in diabetic patients.^{21,22} Although the correlative studies are suggestive, more direct evidence on an active role for MDA in the onset of diabetic complications is needed.¹ Our results showed increased level of plasma MDA in diabetics that may be due to impaired lipid metabolism,²³ or functional change of the erythrocyte membrane following inhibition of SOD that results in superoxide radical accumulation and tissue damage.²⁴ Due to the possible role of hyperglycemia in the lipid peroxidation process, we evaluated the correlation of HbA1c level as an indicator of glycemic control with MDA in diabetic patients. Our results showed a positive correlation between these parameters that confirmed the result of earlier studies.²⁵ However, some researchers could not report a positive correlation of MDA with HbA1c in diabetic patients.^{13,23} Higher levels of HbA1c in patients with diabetes can indicate that they have poor glycemic control. We found higher levels of triglyceride and total cholesterol, and lower levels of HDL-cholesterol in patients with type 2 diabetes. Although, an earlier study reported no correlation between MDA and triglyceride levels in diabetic patients,²⁶ we found a positive correlation between plasma MDA and serum triglycerides. Antioxidant enzyme activities vary among different tissues, and environmental factors might affect the enzyme activity only in susceptible organs. Therefore, the activity found in erythrocytes does not

Table 1 - Clinical and chemical characteristics in diabetic and control groups.

Parameter	Diabetic	Controls
Number	30	30
F/M	14/16	15/15
Age (years)	49 ± 7.5	47 ± 9.2
Glucose (mg/dL)	231.6 ± 79.7*	86.4 ± 12.5
Total cholesterol (mg/dL)	199.4 ± 29.5*	166.2 ± 34.6
HDL-C (mg/dL)	35.4 ± 5.2*	42.1 ± 4.8
Triglycerides (mg/dL)	211.5 ± 54.2*	111.3 ± 32.6

* $P < 0.05$ compared to control, HDL-C - high density lipoprotein cholesterol, F - female, M - male

Table 2 - Oxidative stress markers in blood of diabetic patients and controls subjects.

Marker	Diabetics	Controls
GPx activity(U/g Hb)	19.7 ± 4.3*	53.4 ± 18.2
SOD activity(U/g Hb)	955.7 ± 115*	1371.8 ± 232.6
Plasma MDA(μmol/Liter)	1.1 ± 0.26*	0.36 ± 0.13
HbA _{1c} (%)	10.2 ± 1.75*	5.1 ± 0.5

Values are given as mean ± SD, GPx - glutathion peroxidase, SOD - superoxide dismutase, MDA - malonedialdehyde, HbA_{1c} - glycated hemoglobin, *significantly different from control, $p < 0.001$

necessarily reflect the antioxidant defense of the whole organism. Some earlier studies could not find any significant change in GPx activity between diabetics and control.^{27,28} Nevertheless, we found decreased levels of GPx in diabetics that may be an indicator of high ROS production in these patients. The discrepancy in the GPx activity may involve the presence of complications in the sample population, although not every study evaluated the impact of complications on GPx activity.

Our results of SOD showed lower activity of this enzyme in diabetic patients, confirming the result of earlier studies.²⁹ These data support a role for SOD inhibition in diabetic-induced oxidative stress. Further studies are needed to realize how decreased activity of antioxidant enzymes is related to lipid peroxidation in diabetic patients with and without complications.

References

- Niedowicz DM, Daleke DL. The role of oxidative stress in diabetic complications. *Cell Biochem Biophys* 2005; 43: 289-330.
- Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler H, Rosen P, Halliwell B, et al. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. *Diabetologia* 1997; 40: 647-653.
- Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003; 17: 24-38.
- Valeri C, Pozzilli P, Leslie D. Glucose control in diabetes. *Diabetes Metab Res Rev* 2004; 20: S1-S8.
- Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc Diabetol* 2005; 4: 5.
- Kedziora-Kornatowska K, Szram S, Kornatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G. Effect of vitamin E and vitamin C supplementation of antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. *Nephron Exp Nephrol* 2003; 95: 134-143.
- Cinar M, Ulker S, Alper G, Evinc A. Effect of dietary vitamin E supplementation on vascular reactivity of thoracic aorta in streptozotocin-diabetic rats. *Pharmacology* 2001; 62: 56-64.
- Rauscher FM, Sanders RA, Watkins JB 3rd. Effects of coenzyme Q10 treatment on antioxidant pathways in normal and streptozotocin-induced diabetic rats. *J Biochem Mol Toxicol* 2001; 15: 41-46.
- Mekínová D, Chorváthová V, Volkovová K, Staruchová M, Grancíková E, Klvanová J, et al. Effect of intake of exogenous vitamins C, E and beta-carotene on the antioxidative status in kidneys of rats with streptozotocin-induced diabetes. *Nahrung* 1995; 39: 257-261.
- Maritim AC, Sanders RA, Watkins JB 3rd. Effects of alpha-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. *J Nutr Biochem* 2003; 14: 288-294.
- Turk HM, Sevinc A, Camci C, Cigli A, Buyukberber S, Savli H, et al. Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 diabetes mellitus. *Acta Diabetol* 2002; 39: 117-122.
- Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant / antioxidant balance. *Diabetes Metab* 2000; 26: 163-176.
- Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, et al. Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. *Am J Med* 1995; 98: 469-475.
- Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. *Singapore Med J* 2005; 46: 322-324.
- Martín-Gallán P, Carrascosa A, Gussinyé M, Domínguez C. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radic Biol Med* 2003; 34: 1563-1574.
- Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158-169.
- McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocyte (hemocuprein). *J Biol Chem* 1969; 244: 6049-6055.
- Rodríguez-Martínez MA, Ruiz-Torres A. Homeostasis between lipid peroxidation and antioxidant enzyme activities in healthy human ageing. *Mech Ageing Dev* 1992; 66: 213-122.
- The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; 329: 977-986.
- Creager MA, Lüscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation* 2003; 108: 1527-1532.
- Mooradian AD. Increased serum conjugated dienes in elderly diabetic patients. *J Am Geriatric Soc* 1991; 39: 571-574.
- Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, et al. Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. *Am J Med* 1995; 98: 469-475.
- Suckling KE, Jackson B. Animal models of human lipid metabolism. *Prog Lipid Res* 1993; 32: 1-24.
- Freitas JP, Filipe PM, Rodrigo FG. Lipid peroxidation in type 2 normolipidemic diabetic patients. *Diabetes Res Clin Pract* 1997; 36: 71-75.
- Losada M, Alio JL. Malondialdehyde serum concentration in type 1 diabetic with and without retinopathy. *Doc Ophthalmol* 1996-1997; 93: 223-229.
- Kesavulu MM, Giri R, Kameswara Rao B, Apparao CH. Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. *Diabetes Metab* 2000; 26: 387-392.
- Kaji H, Kurasaki M, Ito K, Saito T, Saito K, Niioka T, et al. Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non-insulin-dependent) diabetic women. *Klin Wochenschr* 1985; 63: 765-768.
- Peuchant E, Delmas-Beauvieux MC, Couchouron A, Dubourg L, Thomas MJ, Perromat A, et al. Short-term insulin therapy and normoglycemia. Effects on erythrocyte lipid peroxidation in NIDDM patients. *Diabetes Care* 1997; 20: 202-207.
- Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clin Sci* 1996; 90: 255-260.