OXIDATIVE STRESS AND METABOLIC CONTROL IN TYPE 2 DIABETIC PATIENTS +

الإجهاد المؤكسد و السيطرة السكرية في مرضى داء السكري النوع الثاني

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Abstract:

Objectives: The aim of this study is to investigate some oxidative stress related parameters in type 2 diabetes mellitus (T2DM) and to determine the effect of glycemic control on these parameters.

Methods: Serum lipid peroxidation product (MDA) and glucose levels and the activity of superoxide dismutase(SOD), catalase(cat) and HbA1c ratio in erythrocytes were assessed. In addition serum levels of copper, zinc and iron were also measured using flame atomic absorption spectrophotometer

Results: Erythrocyte -SOD and CAT activities and serum zinc level in type 2 DM(T2DM) were significantly lower than those of the control subjects ($p < 0.001$). MDA level in type2DM was significantly higher than the control subjects ($p < 0.001$). During poor glycemic control ,on the other hand, serum copper showed no significant differences . A statistically negative correlation ($p < 0.01$) was found between MDA generation and SOD, CAT and serum zinc and a significant positive correlation with serum copper and glucose. Serum iron showed no significant positive correlation with MDA in T2DM patients .A statistically negative correlation ($p<0.01$) was found between HbA1c with SOD, CAT and serum zinc. A statistically positive correlation was found between HbA1c with MDA, serum copper and glucose ($p< 0.01$).

Conclusion: There is an association between oxidative stress and metabolic control in diabetic patients.

المستخلص:

هدف الدراسة: الهدف من هذة الدراسة هو التحري عن بعض المؤشرات المتعلقة بحالة الأجهاد المؤكسد لمرضى السكري النوع الثاني ومعرفة تأثير السيطرة السكرية على هذة المؤشرات .

طرق البحث: تم حساب منتج اكسدة الشحوم ومستوى الكلوكوز في مصل الدم ونشاط السوبراوكسايددسميوتيز والكاتاليز والهيموغلوبين(A1c) في خلايا الدم الحمر اضافة الى النحاس والخارصين والحديد في مصل الدم بأستعمال جهازالمطياف الدري ذو اللهب .

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النتائج: نشاط السوبراوكسايد دسميتيز والكاتاليز المتواجد في خلايا الدم الحمر وكذلك خارصين مصل الدم اقل بشكل مؤثر في مرضى السكري النوع الثاني منه بمجموعة السيطرة (P<0.001) .

مستوى منتج اكسدة الشحوم في السكري النوع الثاني اعلى بشكل مؤثر منه بمجموعة السيطرة (P<0.001). عند السيطرة السكرية الضعيفة نجد ان النحاس و الحديد في مصل الدم لا يؤشر الى اختلاف مؤثر .

وجدت علاقه احصائية سالبة (P<0.01) بين منتج اكسدة الشحوم و السوبر اوكساید دیسمیوتیز و كذلك بين الكاتاليز و خارصين مصل الدم . و علاقة موجبة مؤثرة لنحاس مصل الدم و الكلوكوز مع السيطرة السكرية .

حديد مصل الدم اوضح عدم وجود علاقة موجبة مؤثرة مع منتج اكسدة الشحوم في مرضى السكري نوع الثاني.

علاقة احصائية سالبة (P<0.01) وجدت بين الهيموغلوبين أي وان سي مع السوبر اوكساید دیسمیوتیز و الكاتاليز و خارصين مصل الدم.

علاقة احصائية موجبة وجدت بين الهيموغلوبين أي وان سي مع كل من منتج اكسدة الشحوم و نحاس مصل الدم و كذلك الكلوكوز (P<0.01) .

المضمون: هنالك دليل واضح بمصاحبة الاجهاد المؤكسد للسيطرة السكرية في مرض السكري .

Introduction:

There is substantial interest in blood glucose concentrations because of glucose reaction, depending on blood glucose concentrations with amino groups of plasma and tissue proteins(Amadori reaction) to form glycated proteins.[1] These glycated proteins gradually transform nonenzymatically into advanced glycation end products and to result in altered protein function of the affected molecules[2] Research on blood glucose concentrations was facilitated by the identification of glycated hemoglobin (HbA1c) as a biomarker of long-term glucose homeostasis that reflects blood glucose concentrations over the previous 6 to 8 weeks [3]. In epidemiologic studies, this biomarker has the advantage that a single assessment of Hb A1c is suitable to classify individuals according to their long-term blood glucose concentrations [4].

Oxidative stress resulting from increased production of ROS (or their inadequate removal) plays a key role in the pathogenesis of late diabetic complications [5] [6].

In uncontrolled diabetes, the level of superoxide dismutase, the enzyme responsible for inactivating the superoxide radical, which degrades to hydrogen peroxide, which is then converted to H_2O and O_2 by other enzymes (CAT and GPx) [7]. There is also some evidence suggesting that a deficiency in erythrocyte catalase, an enzyme responsible for the removal of H_2O_2,, is associated with increased frequency of diabetes [8] .The aim of this study was to evaluate lipoperoxidation and plasma antioxidant status in patients with poorly controlled type 2 diabetes mellitus

Materials and methods:

Subjects:

This study was conducted on 100 (50 male and 50 female) type 2 diabetes mellitus patients aged between 37 and 65 years old, who were attending private clinics for proper follow up and treatment. The present study was carried out during the period from January 2009 till the end of july 2009.

In addition, 110 healthy subjects (59 males and 51 females) aged between 35 and 67 years old were randomly selected as control group. None of these healthy subjects had infection nor any acute or chronic disease state.

The aims of the tests were explained to the participants/ their families, and an informed consent was obtained before enrollment in the study.

Methods:

About 7 ml venous blood samples were obtained from all patients and control subjects. About 3 ml was added to EDTA anticoagulant tubes for antioxidant enzymes in erythrocytes(superoxide dismutase and catalase) and glycosylated Hb . The reminder was allowed to clot in a clean plain tube for 20-30 minutes at room temperature. The serum was recovered by centrifugation and divided into 2 parts; the first part of serum was transferred into plain tubes which was used for measuring malondialdehyde (MDA) and glucose within 1-3 hours. The rest of serum was transferred into another plastic plain tube for measuring copper, zinc and iron. The tubes were stored at -20°C until analysis.

Biochemical parameters:

Lipid peroxidation product (MDA) in serum was measured by the method of Guidet and Shah (1989) by using Randox kit . Under the acid and heating condition of the reaction, the peroxides break down to form MDA, which complexes with thiobarbituric acid (TBA) to form a colored red compound that can be measured spectrophotometerically at 535 nm.

The superoxide dismutase activity in erythrocytes was carried out by the method of Winterbourn et al (1975) [9]. It depends on the ability of SOD enzymes to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide, which is generated by the reaction of photo reduced riboflavin and oxygen. SOD activity was expressed in unit per gram hemoglobin.

The activity of catalase in erythrocytes was carried out by the method of Aibe (1974) [10], which is based on determination of the rate constant (S^{-1}, k) of the hydrogen peroxide decomposition rate. Serum glucose was carried out by the method of Trinder, 1969 by using biolabo kit and HbA_{1c} was using HPLC [11]

Serum copper, zinc and iron were measured using flame atomic absorption spectrophotometer (Pye-Unicam series 2900) by direct aspiration of the serum after being diluted with deionized water (1/10) [12].

Statistical analysis

All results were expressed as mean ± SD. The differences between variables were analyzed statistically by student t - test while the correlation between the data was tested statistically by simple linear regression test by using computer SPSS program. The level for significance was $p<0.05$.

Results

The biochemical characteristics which were investigated in this study for diabetic patients and control groups are presented in Table-1. The serum levels of MDA, glucose and copper as well as erythrocyte HbA_{1c} were higher in diabetic patients compared with the control group. The differences were statistically significant ($p<0.001$). The same table shows that erythrocytes SOD, CAT activities and serum zinc were significantly lower in diabetic patients compared with the control group ($p< 0.001$)

Table 1: Basic biochemical characteristics in all study groups

Parameters	Control n= 110	T2DM n= 100	P-value
S.MDA µmole/L	0.738 ±0.078	1.013 ± 0.15	0.001
Erythrocytes SOD U/g Hb	1442 ± 257	1066 ± 230	0.001
Erythrocytes CAT U/g Hb	309 ± 37.6	240 ± 141	0.001
S. Fe µg/dl	105.03 ±11.28	107.89 ± 11.3	ns
S. Cu µg/dl	102.7± 13.03	118.19 ± 11.73	0.001
S. Zn µg/dl	95.03± 9.42	82.86 ± 13.12	0.001
HbA1c Mol%	4.2±0.86	8.7 ± 1.59	0.01
S. glucose mg/dl	95.7± 12.6	188.7± 48.6	0.001

Values were expressed as mean ± SD.
****,*** significant differences between patients and control groups are (p<0.01 ,p<0.001) respectively.**

In T2DM patients with good metabolic control were those in whom HbA1c level was ≤ 8 % while poor metabolic control HbA1c > 8 % (Ikeda et al 1991) as in Table 2. Patients with poor metabolic control were characterized by significantly high serum MDA, copper and glucose (p < 0.001).

On the other hand, Erythrocytes SOD and CAT activities and serum zinc levels were significantly lower in diabetic patients with poor metabolic control group as compared with good metabolic control diabetic patients (p< 0.001). Moreover, the same table shows there was no significant differences in serum iron levels in two groups (p>0.05).

Table 2: Biochemical parameters in NIDDM patients with respect to metabolic control

parameters	HbA1c%		P-value
	≤ 8	> 8	
No.	48	**52**	
S.MDA µmole/L	0.94± 0.18	1.16 ± 0.21	0.001
Erythrocytes SOD U/gHb	1210 ± 152	946± 220	0.001
Erythrocytes CAT U/gHb	250± 38.6	208±40.2	0.001
S. Fe µg/dl	112.2± 15.5	106± 11.4	0.1
S. Cu µg/dl	115.7±9.58	128.6± 9.8	0.001
S. Zn µg/dl	95±8.94	73.0± 8.55	0.001
S. glucose mg/dl	156.2± 25.6	233± 35.6	0.001

Values were expressed as mean ± SD .

The correlation between MDA with SOD, CAT, iron, copper, zinc, glucose and metabolic control in T2DM patients and control subjects were presented in Table 3.

A statistically negative correlation ($p < 0.01$) was found between MDA and SOD, CAT and serum zinc while a significant positive correlation with serum copper, glucose and metabolic control. Serum iron showed no significant correlation with MDA in T2DM patients.

Table 3: Correlation between MDA and biochemical parameters in type 2 diabetes mellitus patients and control subjects

	MDA	
Parameters	T2DM	Control
SOD	-0.72**	-.190
CAT	-0.581 **	-0.08
Fe	0.012	0.088
Cu	0.420**	0.04
Zn	-0.645**	- 0.03
Glucose	0.398**	0.06
HbA1c	0.710**	0.123

Valuesare expressed as correlation Coefficient (r).
** correlation is significant at $p<0.01$.

The correlation between metabolic control and biochemical parameters, (SOD, CAT, iron, copper, zinc, glucose) in T2DM and control groups were presented in Table 4.

A statistically negative correlation (p0<0.01) was found between HbA1c and erythrocyte SOD, CAT and serum zinc.

A statistically positive correlation was found between HbA1c and serum MDA, copper and glucose ($p< 0.01$).

Table 4: Correlation between HbA1c and biochemical parameters in T2DM and control subjects

	HbA1c	
Parameters	T2DM	Control
MDA	0.710**	0.123
SOD	-0.713**	-0.156
CAT	-0.610**	-0.042
Fe	0.125	0.012
Cu	0.568**	0.195
Zn	-0.775**	-0.421
Glucose	0.784**	0.083

Values expressed as correlation coefficient (r).
*,** correlation is significant at $p< 0.05$ and $p< 0.01$ respectively.

Discussion:

Metabolic disturbances and oxidative stress seem to be tightly related, an improved glycemic control being associated with a lowering of proxidant status [13].

The present study showed a high level of MDA in poor metabolic control patients as compared with good metabolic control diabetic patients. This was demonstrated by other studies [14]-[16]. This change could be attributed to increase free radical production, a phenomenon probably occurred either because of enhanced glycosylation or impaired cellular antioxidant protective systems. This is supported by the highly significant positive correlation between MDA levels and HbA_{1c} in diabetic patients in the study. This finding is consistent with the observations of other workers [14]-[17], while other study showed no correlation [18].

The present study also confirms that diabetic patients exhibit an increased oxidative stress as assessed by serum MDA. Increased oxidative stress in diabetic patients appears to be related to the underlying metabolic abnormalities in diabetes, rather than to the complication of this disease.

This study has illustrated a significant lower of CAT activity in diabetic patient with poor metabolic control than diabetic patients with good metabolic control. This is consistent other studies [15] [16] [19]. This could be explained that poor glycemic control in diabetes is strongly associated with an increase in free radicals. Uncontrolled glucose metabolism may also be the cause of alteration in antioxidant enzymes [19]. This is confirmed by a significant negative correlation of catalase activity with metabolic control. This is consistent with the study of Sozmen et al., (1999)[19]. Also this could be supported by the finding a significant negative correlation between MDA level and CAT activity which was obtained in our study. This is similar with the finding of Kesavulu et al., (2001) [20].

In the current study, the effect of metabolic control on serum zinc levels showed a significant decrease in diabetic patients with poor metabolic control as compared with a good metabolic control . This finding was also reported by several other studies [21] [22] and was not similar with the study of Zargar et al. (2002) [23]. These observations could be explained that metabolic control is responsible for excretion of zinc and disturbance of carbohydrates metabolism [24].

This finding is further confirmed by the significant negative correlation obtained between serum zinc level and metabolic control. This is in agreement with finding of several studies [21] [22]

In regards to metabolic control, serum copper have shown increases in patients with poor metabolic control as compared with the good metabolic control. This could be explained due to effect of metabolic control in regulation of glucose metabolism. In contrast to this study finding, other has not observed alteration [23]. Also, in this study there was a significant positive correlation between HbA_{1c} and Cu levels. This finding confirms that serum Cu levels alter with poor metabolic control.

This study also has revealed a significant positive correlation of serum copper with the production of MDA, and serum copper had a significant negative correlation with SOD activity. These findings further confirm that alteration of serum copper levels is due to oxidant/antioxidant imbalance in diabetic patients in this study. From this study, it could be concluded that there is an association between oxidative stress and metabolic control in diabetic patients.

<u>References:</u>

1. Brownlee M,Cerami A,Vlassara H , "Advanced glycosylation endproducts in tissue and the biochemical basis of diabetic complications" *N Engl J Med* 1988;318:1315-21.
2. Lyons TJ ," Lipoprotein glycation and its metabolic consequences". *Diabetes* 1992;41(suppl):67-73
3. Goldstein DE,Parker KM,England JD , "Clinical application of glycosylated hemoglobin measurements", *Diabetes* 1982;31(suppl):70-80

4. Nathan DM,Singer DE,Godine JE,Harrington CH,Perlmuter LC, "Retinopathy in older type II diabetics. Association with glucose control". *Diabetes* 1986;35:797n801.
5. Brownlee M. ,"Biochemistry and molecular cell biology of diabetic complications", *Nature,* 2001 ; 414 : 813 – 820 .
6. 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 Metab* Res Rev. 2001; 17:189-212.
7. Hartnett ME, Stratton RD, Browne RW, Rosner BA, Lanhan RJ , Armstrong D, " Serum Markers of oxidalive stress and severity of diabetic Retinopathy", *Diabetes care* , 2000 ; 23: 234-240 .
8. Goth L, Lenkey A, Bigler WN, " Blood catalase deficiency and diabetes in Hungary", *Diabetes Care*, 2001;24:1839- 1840
9. Winterbourn CC, Hawkins ER, Brian M & Carrel RW, " The estimation of red cell superoxide dismutase activity" , *J. Lab.Clin .Med.* 1975; 85(2): 337-341 .
10. Aebi H . Catalase In , Berg mayer HU, " Methods of enzymatic analysis" . New *York : Weinheim Academic Press* , 1974 , 647 – 83.
11. John WG.," Haemoglobin A1c: analysis and standardization", *Clin Chem Lab Med* 2003; 41:1199-212.
12. Whiteside PJ.. *Pye Unicam atomic absorption data book 2nd edition Pye Vnicum Ltd. England* (1976) .
13. Wierusz-Wysocka B, Wysocki H, Zozulinska D, Wykretowicz A, Kazmierczak M., " Metabolic control quality and free radical activity in diabetic patients", *Diabetes Res Clin Pract,* 1995, 27,193-197.
14. Pasaoglu H, sancak B, Bukan N., "Lipid peroxidation and resistance to oxidation in patients with type2 diabetes mellitus", *Tohoku J. wxp. Med* . 2004; 203:211-218.
15. Lodovici M, Bigagli E, Bardini G, Rotella C, Lipoperoxidation and antioxidant capacity in patients with poorly controlled type 2 diabetes . Toxicol Ind Health. 2009 ;12(2):121-7.
16. Whiting PH, Kalansooriya A, Holbrook I, Haddad F, Jennings PE., "The relationship between chronic glycaemic control and oxidative stress in type 2 diabetes mellitus". *Br J Biomed Sci.* 2008;65(2):71-4
17. Indran M, Rokiah P, Chan SP, Kuppusamy UR., " Alteration of lipid peroxidation and antioxidant enzymes in young Malaysian IDDM patients" . *Med J. Malaysia* . 2004; 59:106-70 .
18. Vessby J, Basu S, Mohsen R, Berne C, Vessby B." oxidative stress and antioxidant status in type 1 diabetes mellitus ". *J. Intern Med* . 2002; 25(1): 69-76
19. Sozmen B, Delen Y, Girgin FK, Sozmen EY., " Catalase and paraoxonase in hypertensive type 2 diabtes mellitus : correlation with glycemic control clin ". *Biochem* . 1999 ; 32(6): 423-427 .
20. Kesavulu MM, Rao BK, Giri R, Vijayao J. Sabramanyam G, Apparao C., "Lipid peroxidation and antioxidant enzyme status in type 2 diabetes with coronary heart disease" . *Diabetes Res. Clin Pract.* 2001 ; 53(1): 33-9 .
21. Roussel A, Kerkeni A, Zouari N, Mahjouh S, MatheauJ Anderson Ra., "Antioxidant effects of zinc supplementation in Turkish with type 2 diabetes mellitus". *J of the Amer Coll of Nutri* 2003; 22: 316-321.

22. Al-Maroof RA, Al-Sharbatti SS., "Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics", *Saudi Med J* 2006;27(3):334-350.
23. Zargar AH, Bashir I, Masoodi SR, Bashir A, Laway BA, Wani AI, Dar FA , "Copper, Zinc and Magnesium levels in type-1 diabetes mellitus". *Saudi Med J.* 2002;23():539-542.
24. Quilliot D, Dousset B, Guerci B, Dubois F, Drouin P, Ziegler O., " Evidence that diabetes mellitus favors impaired metabolism of zinc, copper , and selenium in chronic pancreatitis" . *Pancreas* . 2001; 22(3): 299-306