Relation of Oxidant-Antioxidant Status with Glycemic control in type 2 diabetic patients

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Abstract:

In diabetes mellitus, persistence of hyperglycemia was reported to cause increased production of oxidative parameters, and decreased antioxidant status. Therefore, the present study considered to evaluate the effect of glycemic control on oxidant-antioxidant status in type 2 diabetic patients. The study examined 145 type 2 diabetic patients, who attend to the National Diabetic Center (Al-Mustansiyriah University). Patients were divided in two groups, as well glycemic controlled (group1[75 patients]) ($HbA_{1c} < 7\%$, and FSG < 110 mg/dl) and poorly controlled (group2 [70 patients]) (had $HbA_{1c} \geq 7\%$, and FSG $\geq$ 110). All patients were reported in the morning after overnight fast, and underwent physical examination and laboratory tests. Height and weight were noted for Body Mass Index (BMI), it was calculated as weight $(Km)/height^2 (m^2)$.

Laboratory evaluations consisted of measuring fasting serum glucose (FSG), glycated hemoglobin (HbA_{1c}), total cholesterol (TC), triglycerides (TG), malondialdehyde (MDA), erythrocyte-Glutation (GSH), ascorbic acid (AA), uric acid (UA), and cerulopalsmine (CP). The results indicate that fasting serum FSG, HbA_{1c}, (MDA), (TC), and (TG), were increased significantly (P<0.01 for FSG, HbA_{1c}, MDA; and P<0.05 for TC, and TC) in poorly controlled subjects comparing to well controlled. Instead of that there is significant increase in (CP) (P<0.05) in poorly controlled patients. On the other hand there is significant decreased in (GSH, (AA), (UA) (P<0.01 for GSH, AA; and P<0.05 for UA) in poorly controlled compared to well controlled diabetic patients. Correlation analysis showed a significant positive correlation in serum HbA1c-MDA, GSH-AA (P<0.01; and P<0.05 respectively) in both group, while there is only positive correlation in HbA1c-FSG (P<0.01) in well controlled patients, and positive correlation in HbA1c-TC in poorly controlled patients. On the other hand, there is a significant negative correlation in MDA-GSH, MDA-AA, and MDA-UA in both studying group. The present study concludes the increased risk of oxidative stress manifested by increased plasma MDA, and decreased antioxidant levels in poor glycemic control. The study therefore suggests, the estimation of antioxidants levels with other routine investigations may be useful in reduce of the oxidative stress in diabetics.

Keywords: Glycemic control.Type2 diabetes.Oxidative stress.

الخلاصة:

افادت دراسات عديدة ان ارتفاع السكر بالدم للمرضى المصابين بداء السكر من نوع الثاني يؤدي الى زيادة معلمات الاكسدة وتراجع نسبة مضادات الاكسدة. لذلك اجريت هذه الدراسة لتقييم مدى تاثير سيطرة نسبة السكر في الدم لمرضى النوع الثاني على معلمات الاكسدة ومضادات الاكسدة. شملت هذه الدراسة 145 مريض مصاب بداء السكر من النوع الثاني من المراجعين للمركز الوطني لعلاج وبحوث السكري/ الجامعة المستنصرية حيث تم تقسيم المرضى الى مجموعتين المجموعة الاولىG1 ذات السيطرة الجيدة (75 مريضا)(نسبة السكر بالدم >110 وخضاب الدم المعسل>7٪) والمجموعة الثانيةG2 ذات السيطرة السيئة(70 مريضا)(نسبة السكر بالدم ≤ 110 وخضاب الدم المعسل ≤7%). اخذت جميع العينات من المرضى بعد صيام 12 ساعة وبعد ان تم فحصهم سريريا ومختبريا. تم حساب معامل كتلة الجسم عن طريق اخذ الوزن والطول(الوزن على مربع الطول بالامتار).اجريت للمرضى التحاليل التالية (مستوى السكر بالدم, خضاب الدم المعسل,الكولسترول,الدهون الثلاثية,malondialdehyde(MDA),erythrocyte–Glutation(GSH),حامض الاسكوربكِ,حامض اليوريك و ceruloplasmine. اظهرت النتائج ان هناك زيادة معنوية في مستوى كل من (السكر في الدم,خضاب الدم المعسل,malondialdehyde (MDA),الكولسترول والدهون الثلاثية) حيث كانت هناك زيادة معنوية عالية جدا

(P<0.01)في كل من مستوى السكر بالدم ,خضاب الدم المعسل, malondialdehyde (MDA) وكانت هناك زيادة معنوية (P<0.05) في كل من الكولسترول والدهون الثلاثية في المجموعة ذات السيطرة السيئة اذا ماقورنت بالمجموعة ذات السيطرة الجيدة بالاضافة الى ذلك كانت هناك زيادة معنوية في(P<0.05)ceruloplasmine(CP).من ناحية اخرى كان هناك انخفاض معنوي في erythrocyte-GSH وحامض الاسكوربA.Aب, حامض اليوري U.A (P<0.01)في كل من GSHوحامض الاسكوربك A.A و 0.05 < P في حامض اليوريك (U.A) في المجموعة ذات السيطرة السيئة اذا ما قورنت بالمجموعة ذات السيطرة الجيدة واظهرت تحاليل الارتباط وجود علاقة معنوية موجبة بين ال HbA1C–MDA (P<0.01), A.A-GSH(P<0.05) في المجموعتين بينما كانت هناك علاقة معنوية موجبة واحدة فقط في مرضى السيطرة الجيدة بين (سكر الدم – خضاب الدم المعسل)(P<0.01).وعلاقة موجبة واحدة بين الكوليسترول – خضاب الدم المعسل في مرضى السيطرة السيئة .من ناحية اخرى كانت هناك علاقة معنوية سالبة بين MDA- GSH, MDA- AA ,MDA-U.A في كلا المجموعتين.

نستنتج من هذه الدراسة ان زيادة خطر اجهاد الاكسدة وانخفاض مستوى مضادات الاكسدة يكون واضحا بزيادةMDA عند مرضى السكري ذوي السيطرة السيئة لمستوى السكر في الدم. من خلال هذه النتائج نقترح الاعتماد على قياس نسبة مضادات الاكسدة كجزء من تحاليل الروتينية التي قد تكون مفيدة في الحد من اجهاد الاكسدة في مرضى السكري.

Introduction:

The main cause of morbidity and mortality in both type 1 and type 2 diabetes (T1DM, T2DM) is the development of micro- and macrovascular complications. Several studies have shown a direct relation between glucose exposure, as measured by hemoglobin A1c (HbA1c) level, and complications of diabetes [1]. The molecular mechanisms that explain this relation between hyper-glycemia and diabetic compli cations have not been fully elucidated. A suggested common pathway by which hyperglycemia leads to these complications is the formation of reactive oxygen species (ROS) by glucose overload in the mitochondria. This could lead to vascular damage through several molecular mecha nisms[2]. However, HbA1c only explains around 25% of the variation in risk of developing complications [3]. suggesting the contribution of other factors. It has been suggested that glycemic variability contri butes to diabetes complications via forma tion of ROS [4]. Shadia *et. al.* [5]. showed that persistence of hyperglycemia in diabetes mellitus is the cause to increased production of oxidative parameters as well as defects in antioxidant defense system. A strong rela tionship between glycemic variability and oxidative stress in poorly regulated type 2

diabetes (T2DM) on oral medication has been reported[6]. Free radicals are very reactive chemical species, can cause oxidative injury to the living organisms by attacking the macromolecules like lipids, carbohydrates and nucleic acids. Under normal physiological conditions, there is a critical balance between the generation of oxygen free radicals and antioxidant defense systems used by the organisms, to deactivate and protect themselves against free radical toxicity[7]. Impairment in the oxidant/ antioxidant equilibrium creates a condition known as oxidative stress. It is known to be a component of molecular and cellular tissue damage mechanism in a wide spectrum of human diseases[8]. Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to non-enzymatic glycosyla tion (placation), autooxidation of glycation products, but also changes in the tissue content and activity of the antioxidant defense systems[9]. Oxidative stress as well as defects in antioxidant defense systems are recognized as causative factors for the development of major diabetic compli cations[10]. A variety of natural antioxidants exist to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. One group of these antioxidants is enzymatic (intracellular), which include

super oxide dismutase, glutathione peroxidase and catalase. In addition to enzymatic antioxidants, the major natural antioxidants, most of them derived from natural sources by dietary intake are vitamin A, vitamin C and vitamin E and carotenoids. Also, numerous small molecules are synthesized or produced within the body that has antioxidant capacity (e.g. glutathione and uric acid), and some metal chelator such as ceruloplasmine and transferrine [11-13]. Lipids, the substrate under greatest attack by free radicals, have been the most extensively studied and in diabetic patients are of special interest because their hyperlipidemia (excess substrate) is considered to be a significant risk factor for the development of microvascular complications [14]. Peroxida tion of fatty acids produces aldehydes such as thiobarbituric acid reacting substances (TBARS).The most commonly measured TBARS is malon dialdehyde[15]. A number of studies have evaluated oxidative stress and antioxi dant status in type 2 diabetic patients[9,11]. this study was planned to evaluated serum lipid peroxidation marker, malondiald ehyde (an oxidant) and selected antioxidant (erythrocyte-Glutation (GSH), Ascorbic acid (AA), uric acid (UA) and ceruloplasmine (CP) in relation to glycemic control in type 2 diabetic patients.

Patients and Methods:

The current study included 145 previously diagnosed type 2 diabetic patients; their mean age was 47.7 ± 10.68 years, who attend to the National Diabetic Center (Al-Mustansiriah University). Study cases were divided in two groups according their mean hemoglobin (HbA_{1c}), and fasting serum glucose (FSG) values, as good controlled (group1 [75 patients]) (had $HbA_{1c} < 7\%$, and FSG < 110 mg/dl) and poorly controlled (group2 [70 patients]) (had $HbA_{1c} \geq 7\%$, and FSG $\geq$ 110) patients with DM, both group were matching with age,

body mass index (BMI). The selected patients with diagnosed cardiovascular disease, kidney disease, liver disease, and those who smoked were excluded from this study; none of the patients were taking vitamin or mineral supplement shortly before or during the study. All patients were reported in the morning after overnight fast, and underwent physical exami nation and laboratory tests. Height and weight were noted for Body Mass Index (BMI), it was calculated as weight (Kilogram)/height^2 (meter^2).Laboratory evaluations consisted of measuring fasting serum glucose (FSG), glycated hemog lobin (HbA_{1c}), total chole sterol (TC), triglycerides (TG), malon dialdehyde (MDA), erythrocyte-Glutation (GSH), ascorbic acid (AA), uric acid (UA), and cerulopalsmine (CP). Glucose level was determined using kits supplied by (Randox, UK). Total cholesterol TC, Triglycerides were determined using kits from (spinreact, Spain).Uric acid was measured by enzy matic colorimetric assay using kits supplied by (Randox, UK). Hemoglobin A_{1c} program intended for the determination of Glycated hemoglobin ($A1_c$) in human depended on high performance liquid chromatography and who supplied by Variant Company, USA. Plasma malondiald yhide was measured according to the method described by Shah and Walker [16]. using anticoagulant tubes provided with EDTA.Estimation of erythrocyte glutathione was based on the method described by Butler et al [17]. using anticoagulant tubes provided with acid – citrate-dextrose (ACD). Measurement of whole blood ascorbic acid was done according to the method described by Tetiz et al,[18]. using anticoagulant tubes provided with heparin. Serum ceruloplasmin was measured according to Mancini method[19] by using single radial immune diffusion (RID) endoplates; supplied by Biomegreb, Tunisia.

Statistical analysis:

Data were analyzed using computer facility-the available statistical packages of SPSS-11 (statistical packages for social sciences-version 11.0). Data were present in simple measures of Mean±SD. The significance of difference between quantitative variables was tested using student t-test for comparing between two means of independent groups. P value equal and less than 0.05 was used as the level of significance, and P value equal and less than 0.01 was used as the level of a highly significant.Pearson correlation coeffi cient is significant at the 0.05 level (2-tailed)

Results:

The current study showed that there was a highly significant increased (P<0.001) in fasting serum glucose FSG,glycated hemoglobin (HbA$_{1c}$), and malondialdehyde (MDA) in poorly controlled diabetic patients group G2 comparing to well controlled diabetic patients G1(189.91±22.58 vs 103.3±16.23; 9.1 ± 1.2 vs 6.01± 0.9; 8.9±1.1vs 6.8±0.6) respectively; although,

there was a significant increased increase (P<0.05) in total cholesterol (TC),and trigly cerides (181.88±6.19 vs 164.62±5.2; 198.36±9.07 vs 160.31 ±4.1) respectively. Instead of that; there was significant increase in Ceruloplasmine levels (CP) (P<0.05) in poorly controlled patients. On the other hand, there was a highly significant decreased in (erythrocyte-GSH, ascorbic acid (AA) in poorly controlled diabetic patients compared to well controlled diabetic patients (41.7±5.04 vs 67.18±10.13, and 1.11±0.1 vs 1.71±0.2) correspondingly; and there was significant decreased (P<0.05) in uric acid (UA)(3.63±1.05 vs 5.05±1.1). Correlation analysis showed a highly significant positive correlation in between HbA1c and FSG (P<0.01) only in well controlled patients group, and significant positive correlation (P<0.05) between HbA1c and TC only in poorly controlled group. As well as, there is a significant positive correlation between HbA1c with MDA, and erythrocyte-GSH with AA in both group. On the other hand, there was a significant negative correlation in MDA-GSH, MDA-AA, MDA-UA in both studying group.

Table-1: Clinical characteristics and parameters of type 2 DM according to glycemic control and healthy subjects.

Clinical characteristics	Type 2 DM		P value
	G1	G2	
No.	75	70	-
Age (yrs)	47.6±10.3	48.01± 12.4	N.S
Sex (m:f)	(36: 37)	(35: 38)	N.S
BMI (kg/m^2)	26.95±5.6	27.33±3.5	N.S
Duration (yrs)	7.98±0.8	10.1± 1.2	<0.05*
FSG (mg/dl)	103.3±16.23	189.91±22.58	<0.0001**
HbA1$_c$	6.01± 0.9	9.1 ± 1.2	<0.001**
MDA (µmol/L)	6.8±0.6	8.9±1.1	<0.001**
Erythrocyte-GSH (µmol/L)	67.18±10.13	41.7±5.04	<0.0001**
A A (mg/dl)	1.71±0.2	1.11±0.1	<0.01**
UA (mg/dl)	5.05±1.1	3.63±1.05	<0.05*
CP (mg/dl)	43.4±7.47	51.4±9.59	<0.05*
TC (mg/dl)	164.62±5.2	181.88±6.19	<0.05*
TG (mg/dl)	160.31 ±4.1	198.36±9.07	<0.05*

Data are mean ± SD, *$P \leq 0.05$ was considered significant,
**$P < 0.01$ is a highly significant. N.S non significant.

Table-2: Correlation between parameters in type 2 DM according to glycemic control.

Parameters	G1	G2
	r	r
HbA1c-FSG	0.62**	0.16
HbA1c-TC	0.19	0.44*
HbA1c-TG	0.15	0.23
HbA1c-MDA	0.42*	0.52**
MDA-GSH	-0.71**	-0.77**
MDA-AA	-0.78**	-0.69**
MDA-UA	-0.40*	-0.44*
GSH-AA	0.47*	0.51*

*correlation is significant at the 0.05 level (2-tailed)

**correlation is a highly significant at the 0.01 level (2-tailed)

Discussion:

Diabetes mellitus has been known to be a state of excess generation of free radicals which are contributed by several mechanisms, including hyperglycaemia and a defective antioxidant status, which causes oxidative stress [11]. The data of the present study revealed increased levels of lipid peroxidation marker described as (MDA) and a depleted level of the antioxidant status in poorly controlled type 2 diabetics, in favour of an oxidative stress in such patients. These results were in agreement with those of previous studies [5,11], which demonstrated a strong association between poor glycaemic control and the depletion of the protective antioxidant defense mechanisms in diabetes mellitus. But others [20, 21] failed to observe lipid peroxidation association with glycemic control. This discrepancy may result from the differences in disease duration and / or the age of subjects under evaluation, since the levels of HbA1c have been age dependent and disease duration related [22]. In the present study, all the diabetic patients who are poorly controlled observed hyperlipidaemia, which

was in agreement with the findings of other studies [23,25]. These findings are not surprising, because long-term hypergly caemia causes generalized vascular endo thelial damage, which reduces functional lipoprotein lipase, leading to an increase in TC, TG and a decrease in HDL-C.[25] Several studies suggested that improvement in glycemic control might reduce both microvascular and macrovascular compli cations of diabetes [26,27]. Other reports postu lated a reduction in oxidative stress achieved by optimal glycemic control [28,29]. Malondial dehyde is one of the lipid peroxidation products frequently used to determine the oxidant/antioxidant balance in diabetic pa-tients[30,31]. In agreement with other finding [32], the present study demonstrated that well glycemic control patients had lower MDA level compared to poor-glycemic control group, which may confirm the role of glycemic control to decrease levels of lipid peroxidation by-products, even when these levels were not normalized. It was suggested that hyperglycemia enhance the generation of free radicals through several biochemical pathways (non-enzymatic glycation, polyol

pathway, and glucose autoxidation) [29]. Free radicals can result in consumption of antioxidant defenses and increase susceptibility to lipid peroxidation [29]. Hyperglycemia in diabetes can generate free radicals, and hydrogen peroxide, which cause membrane lipid peroxidation and osmotic fragility in human red blood cells (RBCs). Mechanisms that have been proposed to explain the glutathione effect include the removal of species that initiate lipid peroxidation, scavenging of radicals, scavenging of peroxy radicals and maintenance of membrane protein thiols [33]. Thus GSH may be exhausted through FRs scavenging and forming of oxidized glutathione (GSSG), which is consistently being reduced by glutathione disulfide reductase (GSSG reductase).Consistent with other findings[34] good glycemic control had significantly higher level of Ery–GSH compared to poor glycemic control, which support the hypothesis that glycemic control has a potential role to restore GSH depletion. Although, Hatice *et*al[31]. explain that hyper glycemic conditions, glucose is preferentially used in the polyol pathway that consumes NADPH which is necessary for GSH regeneration by the glutathione reductase enzyme. Hyperglycemia is therefore indirectly the cause of GSH depletion. As GSH is an important molecule, its depletion leads to the increase of oxidative stress. Another finding in the present study showed that well-glycemic control had significant higher level of AA compared to other diabetic group, which support the hypothesis to the effect of glycemic control to improve antioxidant defense systems [26]. This results Consistent

with other [25, 35]. In addition to that, the results documented the hypothesis that glycemic control could restore depletion of antioxidants in diabetic patients [36]. Firoozrai *et*al[37] clarify that ascorbic acid metabolism is altered in conditions such as hyperglycemia and insulin insufficiency and patients with diabetes have about 30% lower plasma ascorbic acid levels than persons without diabetes. Decrease in active transport of reduced ascorbic acid and uptake inhibition of the oxidized form has observed in hyperglycemia [37]. Uric acid considered one of antioxidants that directly scavenging oxygen radicals, singlet oxygen, oxo-haem oxidants and hydroxyl radicals, another important antioxidant property of uric acid is its ability to inhibit ascorbate oxidation, as well as lipid peroxidation. In contrast to other antioxidant scavenger reactions, the inhibition of ascorbate oxidation and lipid peroxidation provided by uric acid does not involve uric acid oxidation [38]. Obviously G1 had higher level of uric acid compared to G2, which is in somehow related to well glycemic control of group1 compared to the poor glycemic control of G2, no available study for the effect of glycemic control on uric acid, but several studies investigate the antioxidants status including uric acid in uncomplicated diabetics which revealed a significant reduction in uric acid levels patients with short duration of disease and without diabetic complications [39]. Cerulo plasmin (CP) is one of plasma proteins that acts as a potent antioxidant, with different physiological roles include scavenge super oxide anion radicals ($O^{\bullet}{}_2{}^{-}$). Through this ability CP acts as a major extracellular

scavenger [40]. CP levels in our study is linked somehow to the degree of glycemic control, as glycemic state worsen and lead to increase CP levels in diabetic patients. The correlations in this study showed a positive correlation between A1c and FSG demons trated in diabetic G1, was helpful to understand that fasting glycemia provide evidence for the metabolic control achieved by HbA1c[41]. Lack of such correlation in G2 may be due to the disturbance in glycemia state of this group. Although, it is important to recognize the associations between parameters as these do not function in isolation [42]. In the current study poor glycemic control was associated with enhanced oxidative stress as indicated by a strong correlation between glycated hemo globin and marker of lipid peroxidation (MDA) in diabetic patients[5,42]. The positive correlations between Ery-GSH and AA were found in both study groups, this was supported by previous studies [27-29]. These studies have suggested that in diabetes GSH is largely consumed, mainly because of the regeneration of AA, which is extensively oxidized in diabetic patients. Thus existence of such correlation in both study groups provided useful evidence for the measure ment of AA in whole blood to be a better index than plasma. MDA presented in this study as lipid peroxidation by-product was negatively correlated with AA [26],Ery-GSH [28,29], and uric acid in both groups. The present study concluded that these correla tions may reflect a significant increase in lipid peroxidation (MDA) accompanied by a significant decrease in the antioxidant mechanism in poorly controlled group by the inverse relationship between them. As a

result, glycemic control has a potential role to lower lipid peroxdation by-products, and reduce consumption of antioxidants. It may be appropriate to evaluate markers of oxidative stress in addition to routine laboratory assessments in evaluation of type2 DM patients. Antioxidant supplemen tation may be necessary for treatment to reduce oxidative stress for diabetic compli cations protection and amelioration.

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