

Free Radical Scavenging Activity of Gliclazide in Type 2 Diabetic Mellitus Patients

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Abstract

To evaluate the effects of 8 weeks of gliclazide therapy on serum glucose level and serum malondialdehyde (MDA) as a marker of lipid peroxidation in newly diagnosed type 2 diabetes mellitus (T2DM) patients. Thirty newly diagnosed T2DM patients were participated in the study (15 males and 15 females) with mean age of (47.55±8.46) years .A thirty healthy subjects (15 male and 15 females) of matching age were also recruited in the study as control group. Both groups were matched regarding age, sex and BMI. Fasting serum glucose (FSG) concentration was estimated using a kit supplied by Biocon (Germany). Serum MDA was measured using thiobarbituric acid (TBA) chemical assay method. Showed that T2DM patients having a significant rise in marker of lipid peroxidation MDA and FSG before starting treatment as compared with the control group. After 8 weeks of gliclazide monotherapy there was a significant reduction in both FSG and MDA level. Gliclazide having free radical scavenger properties showed from the decrease in lipid peroxidation in addition to its hypoglycemic effects.

Key words: Type two diabetes mellitus, Malondialdehyde, Gliclazide

تأثير عقار الكليلازيد على مقاومة الجذور الحرة في مصلى الدم لدى مرضى السكري من النمط الثاني

الملخص

تقييم تأثير ثمانية اسابيع من العلاج بعقار الجليلازيد (80 ملغم يوميا) على مؤشرات اجهاد الاكسدة ومستوى السكر في مصلى الدم لدى مرضى السكري من النمط الثاني. اجريت هذه الدراسة على 30 مريض حديثي التشخيص بداء السكري من النمط الثاني (15 رجل و 15 امراة) معدل اعمارهم (47.5±8.6) سنة. كما ضمت 30 شخصا أصحاء ظاهرياً استخدمت كمجموعة ضابطة وقد كانت المجموعتان متطابقتان من حيث العمر، الجنس و دليل كتلة الجسم. تم قياس مستوى السكر في مصلى الدم قبل البدء باخذ العلاج بواسطة عدة قياس مستوى السكر في مصلى الدم من شركة بايكون الالمانية بينما تم قياس مستوى المالدندالدهايد عن طريق المعاملة الكيميائية لمادة الثايوباربيجورك اسيد الحامضية. واعيدت الفحوصات للمرضى بعد اعطائهم الجرعة الدوائية البالغة 80 ملغ /يوم ولمدة ثمانية اسابيع من عقار الكليلازيد. وتم مقارنة النتائج في الحالتين. أظهرت حصيلة النتائج ان المرضى المصابين بداء السكري من النمط الثاني لديهم ارتفاعا معنويا في مستوى المالدندالدهايد ومستوى السكر في مصلى الدم قبل البدء بل العلاج مقارنة مع مجموعة الضبط. بعد 8 اسابيع من العلاج الاحادي بعقار الكليلازيد ظهر انخفاض معنوي في مستوى المالدندالدهايد اضافة الى انخفاض السكر في مصلى الدم. استنتجت هذه الدراسة ان لعقار الكليلازيد خصائص مضادة لأكسدة الدهون عن طريق مقاومة الجذور الحرة التي غالبا ماتكون مرتفعة في الاشخاص المصابين بداء السكري من النوع الثاني اضافة لتأثيراته الايجابية على خفض مستوى السكر في مصلى الدم. لذلك يعتبر هذا العقار احد الخيارات الجيدة لمعالجة ارتفاع السكر في الدم لمرضى السكري من النمط الثاني.

كلمات الدلالة: داء السكري، النمط الثاني، المالدندالدهايد،

Introduction

Almost 200 million people worldwide have diabetes mellitus (DM), the vast majority having Type 2 diabetes, and prevalence will increase to over 300 million within the next 20 years (Kim&Lee 2010). T2DM is characterized by defects in secretion and action of insulin, resulting in episodic or constant hyperglycemia of varying severity, and hyperglycemia brings high risk of long-term, severe complications (ADA, 2007) . Whereas it is recognized that control of hypertension, dyslipidemia, central obesity and increase level of reactive oxygen species (ROS) is important in management of hyperglycemia and regarded as key factors for lowering risk of diabetic complications (Ahmed, 2005) .

Malondialdehyde (MDA) is one of the end products of lipid peroxidation a process which occur as deterioration of the polyunsaturated fatty acid in the lipid cell membrane due to free radicals (Del,*et al* 2007). Malondialdehyde can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA .it can be isolated in urine, blood and some tissues .(Karaman .,*et al* 2008). Sulphonylureas (SU) are now widely prescribed for the treatment of T2DM patients. They stimulate the release of insulin from the pancreas. They act by binding to the SU receptors (SUR1) on the pancreatic β cells resulting in the closure of the adenosine triphosphate-sensitive potassium (K^+ ATP) channels and release of insulin in a glucose-independent manner. Among them, glibenclamide and gliclazide are the most popular agents at present (Sharma *et al.*, 2005).

The advantageous property of gliclazide, independent of antihyperglycemic action, is its free radical scavenging activity seen at plasma concentration below the therapeutic range. The most common side effects are hypoglycemia and weight gain. (Leibowitz & Cerasi 1996).

Patients and Methods

Thirty newly diagnosed T2DM patients participated in the study; (15males and 15 females). Their mean age was (47.55 \pm 8.46) years. These participants were clinically examined by the physicians of the clinic and they were put on oral gliclazide (80 mg/day) for eight weeks. Thirty healthy subjects (15 male and 15 female) of matching age, sex, were participated in this study as a control group .All type 1 diabetics Patients excluded from the study, smoker, obese, pregnant and lactating women also excluded from the study. After the clinical evaluation, patients taking oral hypoglycemic agents other than gliclazide and those taking drugs that may affect the results of the study such anti-oxidant as vitamin A,C,E and selenium , antihyperlipidemic agents have also excluded.

Regional research committees at College of Medicine and Mosul Health Administration approved the study protocol. The study was a randomized control trial, performed at Al-Wafaa diabetic center in Mosul city from the period of 1/11/2011 through 1/6/2012. The biochemical investigation includes fasting serum glucose (FSG) and serum Malondialdehyde (MDA). About 10 ml of venous blood was drawn, from diabetic patients prior to the initiation and after eight weeks of the drug therapy and then serum was separated by centrifugation at 3000 rpm for 10 minutes and kept frozen at -20 °C to be analyzed later. Samples from healthy control subjects were collected and processed in the same way.FSG was estimated using a kit supplied by Biocon (Germany). Serum MDA was measured using thiobarbituric acid (TBA) chemical assay. Standard statistical methods were used to determine the mean and standard deviation .Unpaired t-test was used to compare the results of various biochemical parameters of diabetic patients with the controls. Paired t-test was used to compare the results of various biochemical parameters

between diabetic patients before and after therapy. P value ≤ 0.05 was considered to be statistically significant (Kirkwood, 1988).

Result

Thirty newly diagnosed T2DM patients were included in this study. Their mean \pm SD age was (47.55 \pm 8.46) years. The patients were followed for eight weeks

after receiving their hypoglycemic agent (Gliclazide). Also, thirty apparently healthy subjects, without drug therapy served as a control group. Their mean \pm SD age was (45.85 \pm 7.26) years. Table (1) shows that the serum levels of FSG and MDA were significantly higher ($p < 0.001$) in type 2 diabetic patients before starting therapy in comparison with the control group.

Table (1):- Comparison of FSG and MDA concentration between control and type 2 diabetic patients before Gliclazide therapy.

Parameters	Mean \pm SD		p-value
	Control (n=30)	Before Gliclazide (n=30)	
FSG (mmol/l)	5.02 \pm 0.54	12.42 \pm 2.08	<0.001
MDA(μ mol/l)	1.15 \pm 0.17	3.19 \pm 0.96	<0.001

Significant at p-value < 0.05 using unpaired t-test

Table (2) shows that after eight weeks of gliclazide therapy, FSG and serum MDA levels although reduced but still there

were highly significant differences ($p < 0.001$) from the control values

Table (2):- Comparison of FSG and MDA concentration between control and type 2 diabetic patients after gliclazide therapy

Parameters	Mean \pm SD		p-value
	Control (n=30)	After Gliclazide (n=30)	
FSG (mmol/l)	5.02 \pm 0.54	9.15 \pm 1.58	<0.001
MDA(μ mol/l)	1.15 \pm 0.17	1.91 \pm 0.64	<0.001

Significant at p-value < 0.05 using unpaired t-test

By comparing the values of FSG and MDA in type 2 diabetic patients before and after therapy, there was a significant

($p < 0.001$) decrease in FSG and serum MDA levels after eight weeks of Gliclazide therapy, as shown in Figure (1).

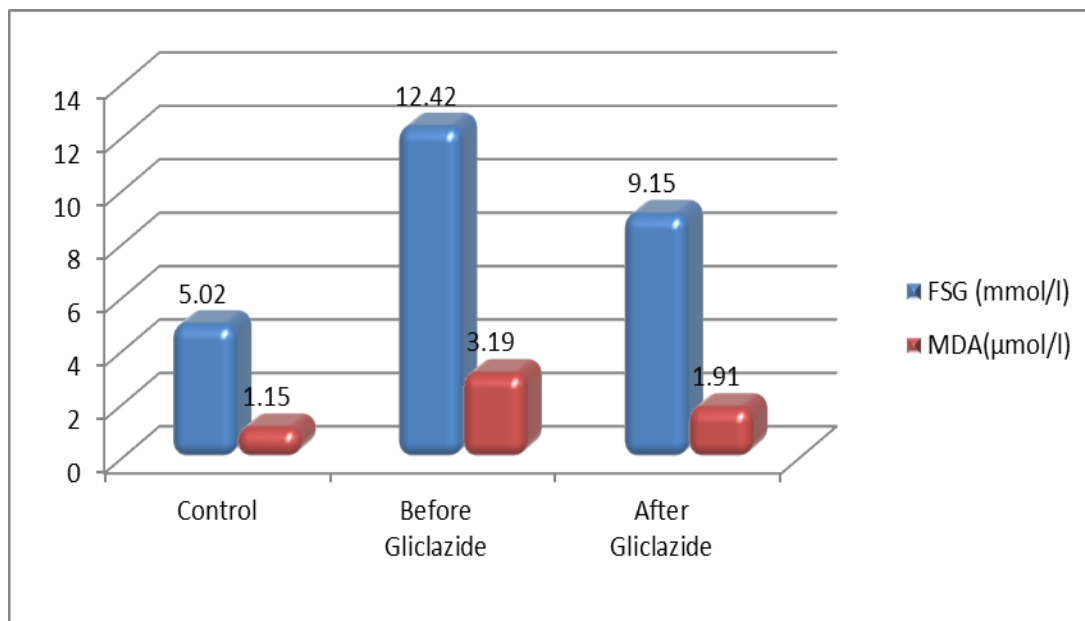


Figure (1):- Effect of Gliclazide therapy on FSG and MDA Concentration in type 2 diabetic patients (pre and post-therapy stages)

Discussion

Diabetes mellitus, a common metabolic disorder resulting from defects in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by glycosuria, polydipsia, and polyuria (Celik, *et al* 2002). During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defense mechanisms imbalance can result in cell dysfunction and destruction resulting in tissue injury (Ceriello, 2000). Free radicals generated during oxidative stress damage the insulin receptors and thereby decrease the number of sites available for insulin action (Kahn, 1994). In the present study, it was observed that the serum MDA and

FSG levels were significantly higher in newly diagnosed T2DM patients as compared to healthy controls. These findings are in agreement with the work of many researchers Pasaoglu *et al.*, (2004) who measured serum MDA and FSG levels in newly diagnosed type 2 diabetic patients. They found that serum MDA and FSG levels were significantly increased in these patients compared to control group. They suggested that autooxidation of glucose may lead to free radical production in diabetic patients which increase lipid peroxidation. Samosaa (2008) was showed an increase in MDA levels in comparison to controls. He suggested that the increase in lipid peroxidation may appear early in T2DM, before the development of secondary complications. Our finding of enhanced lipid peroxidation end product (MDA) provides evidence for the presence of oxidative stress in newly diagnosed T2DM patients. This can be explained by the fact that hyperglycemia in T2DM patients

can increase the oxidative stress by several mechanisms, including glucose autooxidation, nonenzymatic protein glycation and activation polyol pathway (Miller and Britigan, 1997). In agreement with our study results (Türkeli *et al.*, 2008) who reported that serum MDA levels were significantly lower in type 2 diabetic patients treated with gliclazide in comparison to diet group. They showed that gliclazide had an oxidative stress-decreasing effect, apart from its anti-hyperglycemic effect. Agrawal and Srinivasan (2009) reported that T2DM patients treated with gliclazide show a significant reduction in serum MDA and FSG levels after four weeks of drug treatment. This is with agreement with our study. Gliclazide is an oral hypoglycemic agent that belongs to the class of sulfonylureas: basic and clinical evidences suggest that gliclazide works as an antioxidative drug, independently from its ability to reduce hyperglycemia (Picon *et al.*, 2003). The anti-oxidant effect of gliclazide may depend on its specific molecular structure with an aminoazabicyclo-octyl ring grafted onto its sulfonylurea core which is thought to be a radical scavenger and hence responsible for the ability of gliclazide to reduce oxidative stress (Vohnout, 1998).

Conclusion

Basic and clinical evidences suggest that gliclazide works as an antioxidative drug, independently from its ability to reduce hyperglycemia. The availability of a compound that simultaneously decreases hyperglycemia, restoring insulin secretion, and inhibits oxidative stress produced by high glucose seems to be an interesting therapeutics choice for treatment and prevention of vascular complications of diabetes mellitus.

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