

Role of glipizide therapy on oxidative stress parameters in the patient with Type-II diabetes mellitus

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ABSTRACT

Background: Oxidative stress has an important role in the pathophysiology of diabetes mellitus (DM) Type-II. Oxidative stress has an important role in the progression of DM Type-II and its related complications such as retinopathy, neuropathy and many others. The present study was carried out to evaluate the effect of glipizide therapy on oxidative stress parameters in Type-II DM.

Methods: Thirty newly diagnosed diabetes patients were given glipizide therapy on 1st day and continue for 3 months. 30 non-diabetic healthy volunteers served as a control. Plasma malondialdehyde (MDA), superoxide dismutase (SOD) and catalase levels were measured at the time of enrollment and at the end of 3 months of glipizide treatment.

Result: The results are analyzed using paired *t*-test. Plasma MDA was significantly increased, whereas SOD and catalase were significantly reduced in newly diagnosed diabetic patients as compared to control. After 3 months of glipizide therapy, plasma MDA was significantly reduced, whereas SOD and catalase were significantly increased.

Conclusion: Glipizide therapy significantly reduced oxidative free radicals and increased antioxidant mechanism, which reduced oxidative stress, progression DM-II and its related complication.

Keywords: Type-II diabetes mellitus, Oxidative stress, Reactive oxygen species, Glipizide

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that requires lifelong care.¹ Type-II DM is a metabolic disorder of chronic hyperglycemia with disturbances of carbohydrate, protein and fat metabolism due to in defects of insulin secretion, insulin action, or both. Type-II DM is common metabolic problem characterized by hyperglycemia, glycosuria and insulin resistance.¹ The pathogenesis in Type-II DM is such that although the pancreas produces insulin, the body does not utilize the insulin correctly. This is primarily due to insulin resistance peripheral tissue, where the insulin-receptors within the body cells are insensitive to insulin resulting in glucose not readily entering the tissues, thus ultimately leading to hyperglycemia or elevated blood glucose concentrations.^{2,3}

Oxidative stress is defined as a state of imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to cellular damage.⁴ Increased oxidative stress has been demonstrated in various animal models of DM-II as well as in human DM-II.⁵ It is suggested that activation of specific signaling pathways in DM-II leads to overproduction of reactive oxygen species and free radicals including superoxide ions, hydroxyl radicals and hydrogen peroxide. Moreover, the enzymatic and non-enzymatic antioxidant defenses of the body are also exhausted. The resultant oxidative stress consequently leads to vascular injury, atherosclerosis, renal dysfunction, and hypertensive end-organ damage.^{6,7} Consequently, a few definitive markers of oxidative status have gained importance. These biomarkers include Malondialdehyde (MDA) which is a secondary product of

lipid peroxidation and antioxidant enzymes like superoxide dismutase (SOD), which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, and catalase, which further detoxifies hydrogen peroxide.⁸ Increased plasma level of MDA and decreased activity of antioxidant enzymes like catalase and SOD in human DM-II has been demonstrated previously.⁹ Nevertheless, the available data is not conclusive and the association between human blood glucose and oxidative stress remains to be elucidated. Hence, the present study was undertaken to assess MDA, catalase and SOD activities in randomly selected healthy voluntary and DM-II and to study the relationship, if any, between blood glucose and these biomarkers of oxidative stress.

Glipizide is second generation sulfonylureas drugs, which used in the treatment of Type-II DM to reduce blood sugar level.¹⁰ Some study suggest that glipizide, metformin and rosiglitazone reduce some oxidative stress parameters in Type-II DM patient.¹¹ Considering the above facts relationship among glipizide and oxidative stress is complex. Thus, this study is designed to know whether oxidative stress is present or not in DM patients (where other possible factors causing oxidative stress) are absent. If yes, to determine the effect of glipizide treatment on oxidative stress in these patients. If glipizide shows a positive impact on oxidative stress in diabetes patients, hypothesis about additional benefit of glipizide in these patients can be made and tested by further research.

METHODS

Study subjects

The study was conducted at the New Civil Hospital, Surat during the period April 2012 to March 2013. The study was approved by the Institutional Ethics Committee. Informed consent was obtained from the participants.

The individuals participating in this study were divided into these groups: Normal healthy volunteers (controls n=30), Newly diagnosed DM-II patients (n=30), newly diagnosed DM-II patients started on glipizide (n=30) based on inclusion and exclusion criteria.

Methods

This was cross-sectional design study. The study protocol was approved by Institutional Ethics Committee.

Subjects

The study included 30 newly diagnosed DM-II patients never treated previously for DM-II. All patients were between the ages of 45 and 65 years. DM-II was diagnosed by patients symptoms of diabetes plus random blood glucose concentration 11.1 mmol/L (200 mg/dL) a or fasting plasma glucose 7.0 mmol/L (126 mg/dL) b or 2-hr plasma glucose 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance

tested. 30 Type-II DM patients healthy volunteers served as controls. After explaining the study details, informed consent was obtained from all the participants. Patients with cerebrovascular or coronary artery disease, congestive heart failure, hypertension, renal or liver disease and any active viral or bacterial infection were excluded from the study. Smokers, alcoholics, and subjects taking any medication known to affect oxidative stress were also excluded.

Samples

After enrollment, 5 ml venous blood sample was collected in Na-Ethylenediaminetetraacetic acid (1 mg/ml) tubes from each subject and centrifuged immediately at 3000 rpm for 15 mins. The separated plasma was used for estimation of MDA. Cells were washed with normal saline 3 times and red blood cells were subjected to lysis by adding 3 ml ice cold distilled water. Hemolysate was then precipitated by adding 1 ml ethanol and 0.6 ml chloroform. The mixture was mixed thoroughly on the vortex and centrifuged at 3000 rpm for 15 mins. Supernatant was used for estimation of SOD and catalase activity.

Methods

Plasma MDA was estimated as per the method described by Ohkawa et al.¹² 0.5 ml plasma, 0.5 ml of normal saline, 1 ml of 20% trichloroacetic acid and 0.25 ml thiobarbituric acid (TBA) reagent (200 mg of TBA in 30 ml distilled water and 30 ml of acetic acid) were added in a test tube and kept in a boiling water bath at 95°C for 1 hr. The tube was centrifuged at 3000 rpm for 10 mins and optical density of the supernatant was measured in a spectrophotometer at 535 nm. MDA level was expressed in terms of nmol/ml of plasma. SOD activity was measured by method of Marklund and Marklund¹³ with some modifications described by Nandi and Chatterjee.¹⁴ Briefly, 50 µl hemolysate was added to 1 ml of air equilibrated tris-HCl buffer (pH 8.2) in a cuvette and allowed to incubate at room temperature. The reaction was started by adding 10 µL of freshly prepared 2.6 mM the pyrogallol solution (252 mg pyrogallol and 10 µM HCl added to 100 ml distilled H₂O). The rate of increase in the absorbance was recorded for a period of 2 mins, from 1 min 30 sec to 3 mins 30 sec at 420 nm. A 50% inhibition of autooxidation of pyrogallol was measured, and activity of SOD was expressed as U/gHb. Measurement of catalase was done by method described by Sinha.¹⁵ In brief, 0.1 ml hemolysate was added to 1 ml of phosphate buffer (pH 7.0) in a test tube. From 6% H₂O₂ solution, 0.4 ml (800 µM) was then added to it. The reaction was stopped exactly after 1 min, by adding 2 ml potassium dichromate solution (5% potassium dichromate and glacial acetic acid in 1:3 proportions). Test tube was heated for 10 mins in water bath and optical density was measured at 570 nm. Total number of moles of H₂O₂ consumed was determined and catalase activity was expressed in terms of µmol of H₂O₂ consumed/min/mg Hb.

Statistical analysis

Data were expressed as mean±standard deviation. Student's *t*-test was used to assess statistical differences between the groups. Pearson's correlation analysis was performed to study the correlation between parameters and systolic and diastolic blood pressure. Differences were considered as statistically significant at $p<0.05$.

RESULTS

Thirty DM Type-II patients were enrolled in the study. These 30 patients (mean age 52 ± 5 , 24 males and 6 females) received glipizide treatment. Controls were 30 healthy normotensive subjects (mean age 52 ± 5 , 22 males and 8 females) (Table 1).

Fasting blood sugar (FBS) and postprandial blood sugar (PP_2BS) were 96.97 ± 3.50 mg/dL and 146.43 ± 4.08 mg/dL in the control group, respectively. FBS and PP_2BS were 136.27 ± 5.94 mg/dL and 212.2 ± 6.02 mg/dL in the before treatment group respectively (Table 1). After 3 months of glipizide therapy, FBS and PP_2BS were 102.8 ± 5.1 mg/dL and 131.67 ± 4.61 mg/dL. FBS and PP_2BS levels in the treatment groups were higher than the control group, but remained within normal limits (Table 1).

Table 1 shows FBS and PP_2BS levels in patient and control groups. After treatment, FBS and PP_2BS decreased significantly in glipizide treatment group. The mean FBS levels of two patient groups were still higher than the control group but remained within normal limits. * $p<0.05$ vs. before treatment.

Table 2 shows the oxidative stress parameters in diabetics subjects and the effect of glipizide treatment on these parameters.

Before the Type-II diabetic mellitus therapy serum MDA level was significantly raised in diabetes patients comparison to the control group ($p<0.05$). However, after 3 months of glipizide therapy, the mean plasma MDA level decreased significantly from 5.33 ± 0.89 nmol/ml to 4.81 ± 0.96 nmol/ml (Figure 1).

Erythrocyte SOD level, a component of antioxidant enzyme system, was also measured before and after treatment. In Type-II diabetic mellitus patients before treatment, mean SOD activity was significantly decreased in both the groups compared to control group (* $p<0.05$). With 3 months of therapy with glipizide, SOD activity was significantly increased from 1028 ± 139 to 1112 ± 161 (# $p<0.05$) (Figure 2).

Similarly, erythrocyte catalase level was significantly decreased in the Type-II diabetic mellitus patients compared to controls ($p<0.05$). At the end of 3 months of glipizide treatment, catalase level significantly increased from 2037 ± 328 to 2294 ± 318 ($p<0.05$) (Figure 3).

Table 1: General characteristics of the study population.

Variable	Control (n=30)	Glipizide therapy (n=30)	
		Before treatment	After treatment
Age	52 ± 5	52 ± 5	
Gender			
Male	22	24	
Female	8	6	
FBS	96.97 ± 3.50	$136.27\pm 5.94^*$	$102.8\pm 5.1^\#$
PP_2BS	146.43 ± 4.08	$212.2\pm 6.02^*$	$131.67\pm 4.61^\#$

(# $p<0.05$ vs. before treatment)

Table 2: Oxidative stress parameters in diabetics subjects and effect of glipizide treatment on these parameters.

	Control	Glipizide	
		Before treatment	After treatment
MDA (nmol/ml plasma)	2.61 ± 0.64	$5.33\pm 0.89^*$	$4.81\pm 0.96^\#$
SOD (U/gHb)	1192 ± 149	$1028\pm 139^*$	$1112\pm 161^\#$
Catalase (μ moles/mg of Hb/min)	2268 ± 363	$2005\pm 282^*$	$2156\pm 296^\#$

* $p<0.05$ vs. control, # $p<0.05$ vs. before treatment.

MDA: Malondialdehyde, SOD: Superoxide dismutase

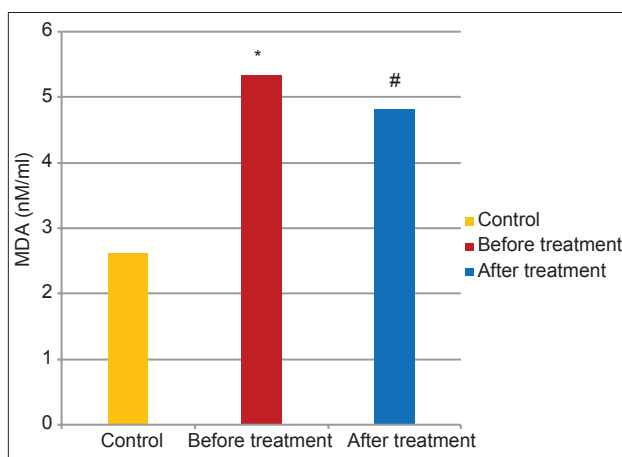


Figure 1: Effect of glipizide treatment on mean plasma malondialdehyde levels in Type-2 diabetes mellitus patients. Mean plasma malondialdehyde (MDA) level was significantly higher compared to controls (* $p<0.05$). After 3 months of treatment with glipizide, mean plasma MDA levels were significantly decreased (# $p<0.05$).

DISCUSSION

Plasma MDA, an index of lipid peroxidation, and SOD and catalase, components of antioxidants systems, in patients

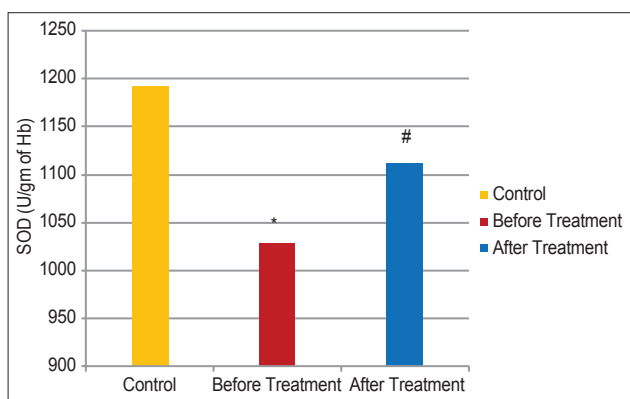


Figure 2: Effect of glipizide treatment on erythrocyte superoxide dismutase (SOD) levels in Type-2 diabetic mellitus patients. Mean erythrocyte SOD levels was significantly higher in compared to controls (* $p < 0.05$). At the end of 3 months of glipizide treatment period, SOD levels were significantly decreased in treatment group (# $p < 0.05$).

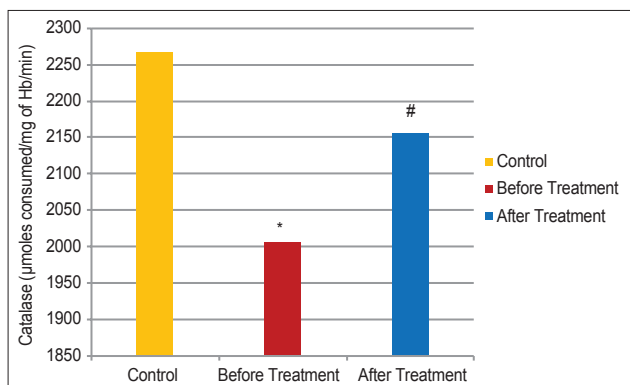


Figure 3: Effect of glipizide treatment on erythrocyte catalase levels in Type-2 diabetic mellitus patients. Mean erythrocyte catalase levels was significantly higher in compared to the controls (* $p < 0.05$). At the end of 3 months of treatment period, catalase levels were significantly decreased in glipizide treatment group (# $p < 0.05$).

of Type-II DM, and the effects of therapy with glipizide on these parameters were examined in this study. The FBS and PP₂ BS decreased within normal range with glipizide therapy. Mean plasma MDA level was significantly higher in patients of Type-II diabetes compared to control group before therapy and it decreased significantly after the treatment of glipizide therapy. Erythrocyte SOD and catalase level were significantly lower in Type-II diabetes patients before therapy in comparison to control group and treatment with glipizide therapy increased the level of both SOD and catalase.

In Type-II DM with increased generation of free radicals and impaired antioxidant defense mechanism, that could be central contribution for reactive oxygen species in the progression of Type-II DM and also associated with

pathological consequences of Type-II DM.¹⁰ In Type-II DM produce hyperglycemia could be associated with overproduction of superoxide. There was one study that oral hypoglycemic, glicazide saw significant effect on oxidative stress and also glicazide saw ability to remove the idoxylic radicals to detoxify the cells and scavenger effect on superoxide ions.¹⁶ Various hypoglycemic agents reduce oxidative stress, indirectly by lowering blood glucose levels and preventing hyper insulinemia, and directly by acting as free radical scavengers.¹⁵

In Type-II DM, increased the level of reactive oxygen species due to the decreased the production of catalase, SOD, and glutathione peroxidase antioxidant enzyme or increased the production of superoxide anion radical hydroxyl radical. So, antioxidant enzymes critically influence the susceptibility of various tissue to oxidative stress and complication of diabetes.¹⁷ MDA is a product of long chain fatty acid peroxidation that accumulates due to increased oxidative stress. MDA further accelerates peroxidation itself by synergizing with free radicals.¹⁸

Glipizide can simultaneously decrease blood glucose, normalized the insulin secretion, and inhibit oxidative stress produced by hyperglycemia seems to be therapeutic prospect for the prevention of vascular complications of diabetes. Glipizide has also been investigated for antioxidant properties. It seems to play a prominent role in scavenging free radicals and restoring antioxidant activities in the tissues of diabetic animals. In this study, we were observed that glipizide therapy maintain blood glucose level in DM Type-II patients. Hence, glipizide therapy reduced the oxidative stress by decreased blood glucose level and preventing hyperinsulinemia and direct acting as free radical scavengers.

CONCLUSION

- Plasma MDA, an index of lipid peroxidation, is significantly increased in DM patients at the end of this study
- SOD and catalase, components of antioxidant system, are significantly decreased in these patients
- Thus it has been observed that in DM patients, oxidative stress is significantly increased
- Glipizide reduced the FBS and PP₂BS level to normal range
- Treatment with glipizide significantly reduced MDA level and increased the levels of SOD and catalase indicating improvement in oxidative stress
- Thus we may speculate treatment with glipizide reduces oxidative stress and may help the patients from developing severe complications of diabetes related to oxidative stress.

However, in this study, we examined the effects of glipizide on oxidative stress for period of 3 month only. As anti-diabetic therapy is generally life long, the long-term

effects of these drugs on oxidative stress need to be further evaluated. Dietary composition can alter the oxidative stress status of an individual and hence the dietary composition of patients may have some impact on their oxidative stress status. Thus to generalized the results of this study, study parameters need to be evaluated in larger population and for longer duration.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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