ORIGINAL ARTICLE

Attenuation of lipid peroxidation and atherogenic factors in diabetic patients treated with gliclazide and metformin

Sujan Banik, Mohammad Salim Hossain, Rita Bhatta, Mariyam Akter
Department of Pharmacy, Noakhali Science and Technology University, Noakhali, Bangladesh

Background: Diabetes is associated with oxidative stress and considered as a major risk factor for cardiac disease. We attempted to investigate the role of oral antidiabetic (OAD) agents gliclazide and metformin in lowering the lipid peroxidation and managing the risk for cardiovascular (CV) complications in diabetic patients in comparison with nondiabetic healthy individuals. **Materials and Methods:** This cross-sectional study was comprised of 150 individuals grouped in three, namely, Group A (n = 60) healthy volunteers, Group B (n = 30) newly diagnosed diabetes, and Group C (n = 60) diabetes treated with OAD. Serum malondialdehyde (MDA), nitric oxide (NO), and Vitamin C were assessed for studying lipid peroxidation status, whereas serum triglyceride (TG) and total cholesterol were monitored as predictors for CV risk. **Results:** We found significantly higher concentrations of MDA and NO levels (P < 0.001) in both groups of patients (Group B and C) in comparison to control group (Group A). Regarding antioxidants, significantly lower concentrations of Vitamin C (P = 0.046) were found in Group B and C compared to Group A. Moreover, there was significant difference exhibited in concentration level of MDA (P = 0.001) and NO (P = 0.015) between Group B and C, whereas difference of Vitamin C (P = 0.147) was not statistically significant. **Conclusion:** Our data confirmed that treatment with gliclazide and metformin significantly reduced the lipid peroxidation accompanied with attenuated levels of serum TGs and cholesterol and suggested that oral hypoglycemic agents have great impact to reduce the oxidative stress and increase the antioxidant status in diabetes.

Key words: Antioxidant, atherogenic factors, cholesterol, lipid peroxidation, oral antidiabetic drugs, type 2 diabetes

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INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, is considered as a major health problem globally. A report by the World Health Organization has estimated that the worldwide prevalence of type 2 diabetes will be increased from 2.8% in 2000 to 4.4% in 2030.[1] The prevalence of this health disorder has been rapidly rising in developing countries like South-East Asia region.[1] Similarly, a recent study showed the trend of higher prevalence of diabetes (7.2%) in Bangladesh.[2] Metabolic syndrome is a component of DM and about 47.0% patients with type 2 diabetes are suffering from metabolic syndrome in Noakhali, Bangladesh.[3]

The presence of metabolic syndrome in patients with type 2 diabetes is associated with a fivefold increase in cardiovascular (CV) risk independent of age, sex, smoking status, and glycated hemoglobin.[4] DM is considered to propagate difficulties with augmented free radical formation.[5] Increased release of free radicals for neutralizing the stress in an individual is the key cause of oxidative stress.[6] These free radicals create the diabetic complications such as retinopathy and neuropathy and also responsible for lipid peroxidation.[7]

For resolving the activity of free radicals, the human body can consume different types of compounds are called antioxidants such as β-carotene, Vitamin E, and

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Address for correspondence: Mr. Sujan Banik, Department of Pharmacy, Noakhali Science and Technology University, Noakhali-3814, Bangladesh.
E-mail: pharmasujan@yahoo.com
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Vitamin C,[8,9] which prevents the process of oxidation. In biological systems, antioxidants can work in a variety of ways, including catalytic removal of free radicals, as scavengers of free radicals or in the form of proteins that minimize the availability of pro-oxidants such as metal ions.

For the treatment of DM, oral sulfonylureas and biguanides are commonly used and they have different mechanisms to reduce blood sugar level. Gliclazide, a member of second-generation sulfonylureas and a common drug for DM, has been reported to act as general free radical scavenger that possesses a sole azabicyclo-octyl ring.[10] Among the biguanides, metformin, the widely used oral antidiabetic (OAD) drug, has been studied for its effect on lipid peroxidation and antioxidant status in dexamethasone-induced type 2 diabetic mice.[11] In our literature review, although there are a few reports about the antioxidant properties of gliclazide and metformin separately, evaluation of these two drugs, in combination, is rare, in terms of lipid peroxidation and antioxidant status; moreover, the role of oral hypoglycemic agents in CV event is a controversial issue because some drugs demonstrated CV benefits, while some antidiabetic drugs (e.g., rosiglitazone and pioglitazone) raised concern about a possible increased CV risk associate with drug use,[12] thus choosing the hypoglycemic agents with cardioprotective effect would be a good candidate for treating type 2 diabetes. Taking all these in consideration, we aimed to assess the impact of both gliclazide and metformin on risk factors for the development of CV disease associated with lipid peroxidation in diabetic patients in Bangladesh.

MATERIALS AND METHODS

Study design and blood sample collection

This cross-sectional study was comprised of 150 individuals (namely 90 diabetic patients and 60 controls) aged between 28 and 70 years. The participants were selected among those attending the Noakhali Diabetic Hospital, Noakhali, Bangladesh. Ethical clearance was obtained from the Ethical Review Board of the institution. Informed consent was obtained from all the participants before their inclusion into the study. The case group consisted of 90 with type 2 DM was categorized into two groups, one group included 30 newly diagnosed patients (Group B) and the other group (Group C) included 60 patients treated with OAD agents for the past 5 years consisted of gliclazide and metformin. The control group (Group A) consisted of sixty healthy nondiabetic individuals (identified by a confirmation test of blood glucose testing) without the history of alcohol and substance abuse or dependence, presence of excessive obesity, and any other diseases. A questionnaire was anticipated to acquire information on the participant's age, sex, height, weight, smoking habits, duration of disease (type 2 diabetes), medication usage, and any other diseases.

Venous blood sample was collected from each of the diabetic patients and controls identified by blood glucose testing, between 8 and 9 am after an overnight fasting. The blood was then allowed to clot at room temperature for 30 min and centrifuged for 15 min at 3000 rpm to extract the serum.[13] The serum was stored at −20°C until analysis.

Laboratory analysis

The collected serum was subjected for measuring the glucose level (fasting and 2 h glucose), total cholesterol, triglyceride (TG), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), and creatinine levels by enzymatic process using colorimetric commercial assay kits (Manufacturer: RANDOX Laboratories Ltd.) and those were routinely analyzed in concern hospital of our study. Atherogenic Index of Plasma (AIP),[14] a predictor of coronary heart disease (CHD), was calculated using the equation: AIP = log (TG/HDL-C). The serum lipid peroxidation product malondialdehyde (MDA), nitric oxide (NO), and antioxidant (Vitamin C) was determined by ultraviolet spectrophotometer according to the methods developed by Satoh,[15] Griess,[16] and Lowry *et al*.,[17] respectively.

Statistical analysis

Descriptive statistics were calculated for all variables using SPSS software package (version 19.0, Armonk, NY, IBM Corp.,). All of the values were expressed as the mean ± standard deviation (SD). Normality was tested using the Kolmogorov–Smirnov test and statistical comparisons were performed by one-way ANOVA followed by Bonferroni *post hoc* test and $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and biophysical characteristics of the study population

The demographic data and biophysical characteristics of diabetic patients and controls are presented in Table 1. Among the participants, 77 were female (51.33%) and 73 were male (48.66%). Mean (SD) age of participants was 42.96 (14.56), 46.76 (14.84), and 45.74 (12.32) years, respectively, for Group A, Group B, and Group C. There was no significant difference ($P = 0.867$) in the mean value of body mass index among the Group A (23.64 ± 5.03 kg/m^2), Group B (27.59 ± 6.41 kg/m^2), and Group C (25.09 ± 4.18 kg/m^2).

Effect of oral hypoglycemic agents on serum glucose

The biochemical characteristics of diabetic patients and controls are presented in Table 2. From statistical analysis,

this study found that the mean levels of fasting blood sugar and 2 h blood sugar were significantly higher ($P < 0.001$) in the serum of both groups of patients (Groups B and C) when compared to the control group (Group A). However, the treatment with gliclazide and metformin reduced the serum sugar level compared to newly diagnosed diabetes as predicted.

Effect of oral hypoglycemic agents on serum lipid profile

The mean levels of total cholesterol and TGs were significantly higher ($P < 0.05$) in the serum of Group B patients when compared to controls (Group A). On the other hand, the mean levels of total cholesterol and TGs were significantly lower ($P < 0.05$) in Group C when compared to Group B.

Effect of oral hypoglycemic agents on the risk of coronary heart disease

Treatment with OAD in diabetic condition ameliorates the risk of CHD by around 5% compared with the newly diagnosed diabetic patients.

Effect of oral antidiabetic on serum malondialdehyde and nitric oxide levels

Our results showed that the mean concentration of MDA and NO levels was statistically higher in both groups of patients ($P < 0.001$) in contrast to control group [Table 3]. Moreover, at a 5% level of significance when the values compared between the both groups of patients (Group B and Group C), Group C treated with OAD showed statistically lower concentrations of MDA and NO levels comparing to Group B.

Effect of oral antidiabetic on Vitamin C level

The analysis of Vitamin C concentrations to compare differences between the patient groups (Groups B and C) and control Group A showed that significantly lower concentrations of antioxidant were found in patients ($P = 0.046$), whereas treatment with OAD was unable to rescue the attenuated values of Vitamin C.

DISCUSSION

Gliclazide and Metformin, in combination, are frequently used to treat the diabetic patients. In this study, we attempted to know the combined role of these oral hypoglycemic agents in reducing the oxidative stress and managing the risk for CV complication in diabetic patients in Bangladesh.

Reactive oxygen species (ROS) such as peroxides, superoxide, hydroxyl radical, and NO have an enormous impact in the pathogenesis of various diseases such as DM, atherosclerosis, cell damage, cancer, myocardial infarct, and hemolytic disorders. As a source of ROS, we determined the level of NO in this experiment. In agreement with other study,[18] our data showed a higher amount of NO in newly diagnosed diabetic patients compared to normal healthy individuals. But the reduce level of NO was found progressively in treated patients with glicazide and metformin concomitantly and indicating the role of OAD in scavenging the free radicals. This result may be attributed to the capacity of gliclazide and metformin to increase the antioxidant activity of erythrocyte catalase, glutathione peroxidase, and glutathione S-transferase enzyme as

Table 1: Demographic data and biophysical characteristics of diabetic patients and control subjects

	Group A (control)	Group B (newly diagnosed)	Group C (treated patients)	*P*
n	60	30	60	
Female/male	26/34	18/12	33/27	
Age (years)	42.96±14.56	46.76±14.84	45.74±12.32	0.650
BMI (kg/m^2)	23.64±5.03	27.59±6.41	25.09±4.18	0.867
Duration of DM (years)	-	-	7±0.93	

The results are expressed as mean±SD. BMI determined with the weight and height of patient. Statistical comparisons were performed by one-way ANOVA followed by Bonferroni a *post hoc* test at 5% level of significance. SD = Standard deviation; BMI = Body mass index; DM = Diabetes mellitus

Table 2: Biochemical characteristics of diabetic patients and control subjects

Parameter	Group A (control)	Group B (newly diagnosed)	Group C (treated patients)	P^a	P^b
Fasting blood sugar (mmol/L)	5.23±0.85	9.46±3.28	9.75±4.73	<0.001*	0.875
2 h blood sugar (mmol/L)	7.57±1.47	14.60±3.94	14.35±6.11	<0.001*	1.000
Total cholesterol (mg/dL)	194.63±52.67	223.54±62.17	172.28±48.10	<0.001*	0.001*
HDL cholesterol (mg/dL)	36.00±10.30	40.77±10.84	31.92±8.13	<0.01*	0.001*
LDL cholesterol (mg/dL)	124.77±44.69	131.00±51.21	101.45±56.86	<0.044*	0.056
Triglycerides (mg/dL)	167.27±85.21	259.38±62.41	169.60±81.10	<0.001*	0.011*
Creatinine (mg/dL)	0.97±0.31	1.08±0.33	1.06±0.85	0.506	1.000
Atherogenic index	0.65±1.16	0.77±0.88	0.73±0.62	0.809	<0.05

The results are expressed as mean±SD. [a]*P* comparing the Group B, and Group C in contrast to Group A; [b]*P* comparing between Group B and Group C; Statistical comparisons were performed by one-way ANOVA followed by Bonferroni a *post hoc* test; *Statistically significant at 95% CI. HDL = High-density lipoprotein; LDL = Low-density lipoprotein; SD = Standard deviation; CI = Confidence interval

Table 3: Serum malondialdehyde, nitric oxide, and Vitamin C levels of diabetic patients and control subjects

Parameter	Group A (control)	Group B (newly diagnosed)	Group C (treated patients)	P^a	P^b
Serum MDA (μmol/L)	2.63±1.63	5.38±1.64	5.09±2.40	<0.001*	0.001*
NO (μmol/L)	15.86±14.95	47.20±70.88	37.92±68.24	<0.001*	0.015*
Vitamin-C (μmol/L)	22.86±12.47	17.21±9.37	17.62±11.93	0.046*	0.147

The results are expressed as mean±SD. [a]*P* comparing between Group B, and Group C in contrast to Group A; [b]*P* comparing the Group B and Group C; Statistical comparisons were performed by one-way ANOVA followed by Bonferroni *post hoc* test; *Statistically significant at 95% CI. MDA = Malondialdehyde; NO = Nitric oxide; SD = Standard deviation; CI = Confidence interval

described by Memisoguliari *et al*. in 2008.[19] We also checked the level of ascorbic acid but did not find any significant changes between the patient groups, whereas both the patient groups had a significantly lower amount of ascorbic acid than that of healthy control. This result suggests that OADs such as gliclazide and metformin have no effect on regulation of ascorbic acid in a diabetic patient. Moreover, ROS is responsible for the lipid peroxidation[20] and MDA, a well-known secondary product of lipid peroxidation,[21] is used for the assessment of oxidative stress in many diseases such as diabetes and vitiligo.[19,22] In this study, we found significantly higher level of MDA in diabetic patients than that in nondiabetic healthy individuals as predicted and reported previously.[23,24] Whereas treatment with OAD agents attenuates the MDA level significantly in treated patients compared to the newly diagnosed diabetic patients. All these results suggest that, along with the capacity of lowering blood glucose level, OAD agents have a distinct role in controlling the oxidative stress in diabetes.

Furthermore, though diabetes is considered as a risk factor for CV disease, the role of oral hypoglycemic agents in the CV event is a controversial issue. We studied the impact of OAD on CV events. Treatment with OAD significantly lowers the level of TG, cholesterol, HDL-c, and LDL-c compared with newly diagnosed diabetes. Different molar ratio (total cholesterol/HDL-c, TG/HDL-c, and LDL-c/HDL-c) was calculated to predict CV risk as described in several reports previously.[14,25,26] Our data suggested that diabetic patients showed significant risk for the development of CV complication as predicted, whereas treatment with OAD in diabetes has a tendency to rescue the risk. We observed about 5% reduction of atherogenic index of patients treated with OAD. We also find a positive correlation ($r = 0.334$) between glucose level and atherogenic index of patients treated with OAD. Our data are in line with data obtained by other researches,[27] who described that metformin has a potential benefit over glipizide on managing CV outcomes in the high-risk patient. On the other hand, gliclazide is considered as a good choice for managing the initial CV risk of patients with diabetes.[28] Moreover, serum level of creatinine was not significantly influenced by the treatment with gliclazide and metformin.

One of the limitations of this study is its small sample size, and the drugs were procured from the different pharmaceutical company. In this research, we have assessed CV factors with a small sample only in relation with cholesterol, and a large-scale study is warranted to get satisfactory data on lowering of CV events by OAD treatment in patient with diabetes.

CONCLUSIONS

Our study revealed that elevated lipid peroxidation in diabetes is associated with the depleted antioxidant status. Treatment with both of gliclazide and metformin significantly reverses the result of lipid peroxidation with the attenuated production of NO. Although OAD treatment showed lower concentrations of serum cholesterol and TG in diabetes significantly, there were no significant changes in molar ratio and atherogenic index for predicting the risk of CV complications. Thus, we concluded that gliclazide and metformin ameliorate the lipid peroxidation in diabetes with a minor change in atherogenic index related with the serum lipids.

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Conflicts of interest

There are no conflicts of interest.

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