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Simvastatin Use in Patients with Type 2 Diabetes Mellitus: The Effects on Oxidative Stress

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ABSTRACT

Objectives: Studies have shown that people with type 2 diabetes mellitus (T2DM) may develop atherosclerosis due to the disturbance in oxidative control and progressive dyslipidemia. Our study aimed to highlight the benefits of simvastatin treatment in improving serum lipids and reducing oxidative damage in patients with T2DM. ***Methods:*** Our randomized control trial included 56 patients with T2DM and dyslipidemia. The participants were on glibenclamide (5mg/day) during the period of the study. The patients were divided into two study groups (groups 1 and 2). Group 1 was the control group and consisted of 31 patients. Group 2 consisted of 25 participants, who were given simvastatin 20mg tablet once daily for 12 weeks. The control group did not receive simvastatin. Both groups were followed-up for measurement of blood pressure, pulse rate, serum lipids, and parameters of oxidative stress. ***Results:*** The simvastatin treated group showed a significant improvement with reduced erythrocyte glutathione compared to the control group ($p<0.001$). This was also associated with a significant reduction in erythrocyte malondialdehyde in the simvastatin treated group compared to the control group ($p<0.001$). Serum lipids reflected a similar improvement in the levels of erythrocyte malondialdehyde. ***Conclusions:*** Our study highlights the beneficial role of simvastatin in improving the degree of oxidative stress in patients with T2DM through its effects on serum lipids and lipid peroxidation.

Patients with type 2 diabetes mellitus (T2DM) develop an imbalance between the production of reactive oxygen species (ROS) and the overall antioxidants defense system.[1] ROS include hydrogen peroxide, hypochlorite ions, hydroxyl radicals, and superoxide anion, which are anions and free radicals.[2] Therefore, excessive generation of ROS in oxidative stress can cause deleterious effects including damage to polyunsaturated fatty acids in membrane lipids, proteins, and DNA and eventually cell death.[3] T2DM is characterized by hyperglycemia, which is a result of defects in insulin secretion or action, or both.[4]

An abnormal lipid profile is commonly seen in T2DM patients and is characterized by abnormal levels of serum lipids and lipoproteins,[5] elevation of triglycerides (TG), and decreased circulating levels of high-density lipoprotein cholesterol (HDL-C) accompanied by an elevation of low-density lipoprotein cholesterol (LDL-C).[6] Evidence suggests there is a close positive association between hyperlipidemia and increased oxidative damage.[7] Simvastatin is a part of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor or statins group of drugs, which act by inhibiting internal cholesterol synthesis inside the body.[8] Inhibition of cholesterol synthesis can be associated with an overall increased production of hepatic LDL receptor (LDL-R) on the cell surface, which promotes efficient uptake and clearance of circulating LDL-C[9] providing a significant improvement in serum lipids. Simvastatin can also improve endothelial cell function, enhance the stability of atherosclerotic plaques and reduce inflammation and, therefore, insulin resistance.[10]

We aimed to highlight the antioxidant effects of simvastatin in treating patients with T2DM and dyslipidemia and determine if the drug could improve serum lipids and reduce oxidative stress.

METHODS

Patients were recruited from the diabetes clinic, Al-Najaf Teaching Hospital, Iraq, between September

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Table 1: Characteristics of the type 2 diabetic patients included in the study.

Parameters	Group 1	Group 2
Number	31	25
Age (years)*	55.6±1.8	57±1.1
Gender (male:female)	14:18	11:14
Diabetes duration (years)	7.8±1.4	6.9±1.3
Fasting plasma glucose (mmol/L)	7.2±1.2	7.8±1.2
HbA_{1c} (%)	6.2±1.2	6.7±1.8

**Data presented as mean ±SD; HbA_{1c}: glycated hemoglobin.*

2010 and December 2011. All participants were informed about the nature of the study and gave informed consent. Table 1 shows the characteristics of the patients who were included in the study. Patients were divided into two groups: group 1 included 31 subjects who were already on oral hypoglycemic medication, glibenclamide 5mg/day (Daonil, Sanofi-aventis, France). This group was not given simvastatin and served as a control group. Group 2 consisted of 25 participants who were also on glibenclamide (5mg/day) but who were administered simvastatin therapy (20mg tablets once daily) for 12 weeks.

Participants were excluded from the study if they used any medications in the last three months that might interfere with the results of serum lipids and oxidative stress parameters (e.g. various vitamins and statins). Patients with renal and or hepatic illness and hypertensive patients with/without antihypertensive medications were also excluded from the study. The study was approved by the Human Ethics Committee, College of Medicine, University of Kufa, Iraq.

Venous blood samples following 10–12 hours of fasting were collected from each participant at the start and at the end of the study (after 12 weeks) to measure plasma glucose, serum lipids, and markers of oxidative stress.

Erythrocyte malondialdehyde (MDA) was measured using the thiobarbituric acid reacting substance method,[11] and the level of erythrocyte reduced glutathione (GSH) was determined using the 5,5'-dithiobis (2-nitrobenzoic acid) reaction.[12] Serum catalase (CAT) activity was determined by ELISA using the Catalase Assay Kit (Sigma-Aldrich, USA), which utilizes the peroxidatic function of CAT for determination of enzyme activity.[13]

Serum lipids (total cholesterol (TC), TG, and HDL-C) were measured chemically using lipid assay kits (BioMerieux, France) and the UV-visible 1650PC spectrophotometer (Shimadzu Europe, France). Serum LDL-C and very low-density lipoprotein cholesterol (VLDL-C) were calculated as per the Friedewald equation.[14] Fasting plasma glucose and HbA_{1c} levels were measured at the diabetes clinic/pathology laboratory of the hospital. Fasting plasma glucose was measured using glucometers and HbA_{1c} was measured using high-performance liquid chromatography (HPLC).

The data were analyzed using SPSS (SPSS Inc., Chicago, USA) version 14 and Microsoft Excel (Office 2007, Microsoft, USA). All values were expressed as the mean± standard deviation (SD). Statistical analysis was performed using a one-way ANOVA followed by paired *t*-test. Pearson's correlations were also performed and significance was set at $p \leq 0.050$.

RESULTS

There was a significant elevation in erythrocyte GSH ($p<0.001$) in the simvastatin treated group (group 2), [Table 2]. This was also associated with a significant reduction in erythrocyte MDA ($p<0.001$). MDA levels increased significantly in the control group

Table 2: Markers of oxidative stress for the two studied groups before and after the study.

Parameters	Group 1		Group 2	
	Before treatment	After treatment	Before treatment	After treatment
GSH (mg/dL)	24±5.8	22±3.6	23±4.6	36±3.8#
MDA (nmol/ml)	12.5±1.5	15.9±2.1*	12.4±1.9	4.8±1.7#
CAT (nmol/min/L)	49±1.2	51±1.6	47±1.7	48±3.9

Values expressed as mean ±SD; GSH: erythrocyte reduced glutathione; MDA: erythrocyte malondialdehyde; CAT: serum catalase.
** Significant p-value <0.001 before and after 12 weeks (group 1)*
 # Significant p-value <0.001 before and after 12 weeks (group 2)

Table 3: Serum lipid profiles in the two studied groups before and after the study.

Parameters	Group 1		Group 2	
	Before treatment	After treatment	Before treatment	After treatment
TC (mmol/L)	6.6±1.2	6.4±0.5	5.7±0.4	4.1±0.3#
TG (mmol/L)	2.8±0.7	2.8±0.6	2.6±0.6	2.1±0.9#
HDL-C (mmol/L)	0.8±0.6	0.8±0.1	0.8±0.2	1.2±0.3#
LDL-C (mmol/L)	4.9±1.3	4.8±0.9	3.9±0.7	2.2±0.5#
VLDL-C (mmol/L)	1.3±0.1	1.2±0.3	1.2±0.3	0.9±0.2#

Values expressed as mean ±SD.
TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol.
 # Significant p-value <0.001 before and after 12 weeks of treatment of group 2.

Table 4: Correlation regression between the parameters of oxidative stress and serum lipids in the simvastatin treatment group.

	GSH	MDA	CAT
TC	0.063	0.059	0.135*
TG	-0.077	-0.115*	0.337*
HDL-C	-0.172*	0.409*	0.095
LDL-C	0.145*	-0.092	0.042
VLDL-C	-0.110*	-0.062	0.268*

** Significant p-value <0.001;*
TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; GSH: erythrocyte reduced glutathione; MDA: erythrocyte malondialdehyde; CAT: serum catalase.

(group 1) at the end of the study, indicating the progressive increase in the degree of lipid peroxidation. Serum CAT showed no significant changes in both the treatment and control groups as shown in Table 2.

The simvastatin treatment group had a significant improvement in all aspects of serum lipid profiles (p<0.001) compared to the control group [Table 3].

Pearson's correlation showed a significant positive association between CAT and TC, TG, and VLDL-C, and between MDA, HDL-C and LDL-C [Table 4]. There was a significant negative association between MDA and TG; this was associated with a significant negative correlation between GSH, HDL-C, and VLDL-C [Table 4].

DISCUSSION

Our study showed a significant improvement in the levels of serum lipids and oxidative stress markers in patients with T2DM and dyslipidemia treated with simvastatin. This manifested due to the significant rise in erythrocyte GSH associated with a significant reduction in the erythrocyte MDA in the simvastatin treatment group (group 2) at the end of the 12-week therapy period. This may be explained by the antioxidant effects of simvastatin, as a result of inhibition of mevalonate pathway causing a significant reduction in the biosynthesis of important intermediates, like isoprenoids, which act as a lipid attachment/anchoring for intracellular signaling molecules, particularly the inhibition of the small GTPase binding proteins whose proper membrane localization and function are dependent on isoprenylation.[15]

However, a significant improvement in oxidative stress was not identified by measuring CAT activity in the simvastatin treated group, which may be due to the presence of other variables that affect CAT activity. Previous studies have reported various changes in CAT activity in chronic diseases like diabetes mellitus.[15,16] Therefore, we recommend increasing the sample size and greater monitoring of CAT activity in future studies.

The significant improvement in the levels of serum lipids in group 2 can be explained by the ability of simvastatin to hinder cholesterol biosynthesis by inhibiting the rate-limiting step in cholesterol synthesis (via the inhibition of HMG-CoA reductase activity).[17] This mechanism lowers the circulating LDL-C and increases their reuptake by their hepatic receptors.[18]

The antioxidant effect of simvastatin was not consistent as there were inconsistent findings when comparing the correlation between markers of oxidative stress and serum lipids. This may be partially explained by the fact that simvastatin can inhibit isoprenoids synthesis rather than inhibit cholesterol biosynthesis.[10,18]

CONCLUSION

Our study proved that simvastatin therapy in patients with T2DM and dyslipidemia caused a significant improvement in the levels of serum lipids and markers of oxidative stress.

Disclosure
The authors declared no conflicts of interest. No funding was received for this work.

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