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Original Article

Biochemistry Section

Effect of Rosuvastatin on Oxidative Stress in Patients with Type 2 Diabetes Mellitus: A Prospective Interventional Study

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is an established risk factor for Cardiovascular Disease (CVD). Oxidative Stress (OS) and inflammation are linked to CVD in Type 2 Diabetes Mellitus (T2DM). Rosuvastatin is a statin of choice in patients at high cardiovascular risk due to its pharmacokinetic efficacy as well as patient safety. There is limited data on the effect of rosuvastatin on OS among Indian subjects with T2DM.

Aim: To assess the effect of rosuvastatin 20 mg for 12 weeks on oxidant and antioxidant status in patients with T2DM.

Materials and Methods: This prospective interventional study was conducted in the Department of Biochemistry at Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India, from March 2018 to February 2019. A total of 24 patients diagnosed with T2DM were included in the study and were administered rosuvastatin tablets (20 mg/day) orally for a period of 12 weeks. The oxidant markers {Malondialdehyde (MDA) and Protein Carbonyl Content (PCC)} and antioxidant markers {Ferric Reducing Ability of Plasma (FRAP) and protein thiols} were analysed spectrophotometrically using standard methods. Paired samples t-test/Wilcoxon's signed-rank test was used as appropriate for the comparison of markers at baseline and after 12 weeks of rosuvastatin intervention. The association between markers studied was assessed using linear regression with the Generalised Estimating Equations (GEE).

Results: The mean age of the study subjects was 48.04±7.96 years. There were 17 male patients (70.8%). Rosuvastatin 20 mg/day showed a lipid-lowering effect {Total Cholesterol (TC), Triglyceride (TG), Low-density lipoprotein cholesterol (LDL-C)}, an increase in the antioxidant and anti-atherogenic Hi-density lipoprotein cholesterol (HDL-C). It also showed a beneficial effect on OS markers as evidenced by a significant decrease in oxidant markers MDA (-9.06%), PCC (-21.2%) ($p<0.05$) and an increase in antioxidant markers FRAP (+9.08%) and protein thiols (+11.8%) ($p<0.05$) 12 weeks after treatment in patients with T2DM. A change in LDL-C was positively associated with a change in MDA and PCC in patients with diabetes postintervention ($p<0.001$).

Conclusion: The findings of the present study suggest that rosuvastatin 20 mg for 12 weeks produces a beneficial effect on CV risk in patients with T2DM. The decrease in OS and the LDL-C levels can thus decrease the formation of oxidised LDL, which initiates the atherosclerotic process.

Keywords: Cardiovascular risk, Lipid profile, Protein carbonyl content, Protein thiols, Statin

INTRODUCTION

The DM is a chronic metabolic disorder and an established risk factor for CVD. Over 65% of patients with diabetes die from some form of heart disease or stroke [1]. The prevalence of diabetes in India is increasing, with the overall reported prevalence of 7.3% and the prevalence of prediabetes being 10.3% or 24.7% [2] based on World Health Organisation (WHO) [3] or the Adenosine Deaminase (ADA) criteria, respectively [4]. The majority of subjects in South Asia and India develop diabetes at a younger age (<50 years) compared to the Caucasian population (>58 years). Additionally, South Asians have been shown to be at a higher risk of developing macrovascular complications like coronary artery and cerebrovascular disease compared to Caucasians [5,6], thus warranting intense therapy directed at controlling DM and reducing risk factors.

The OS and inflammation are important novel risk factors for CVD in T2DM [7-9]. These play an important role in the initiation and evolution of atherosclerosis from endothelial dysfunction to clinical events [7]. Excess reactive oxygen species cause peroxidation of lipids, resulting in the formation of highly reactive aldehydes, such as MDA, which is documented as a primary biomarker for free radical-mediated lipid damage and OS [10]. Oxidative cleavage of the protein backbone and direct oxidation of amino acids result in the formation of protein carbonyls [11].

Under normal conditions, the effect of oxygen-free radical production is largely nullified by a network of antioxidant defense systems. Antioxidants are substances that significantly delay or inhibit the rate of oxidative damage to target molecules and mainly include enzymatic and non enzymatic components [12]. Enzymatic antioxidants comprise Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Glutathione Reductase (GR), whereas non enzymatic antioxidants include low molecular weight compounds such as reduced Glutathione (GSH), albumin, bilirubin, Uric Acid (UA), selenium, vitamins C, E, and carotenoids [12]. Thiols are involved in a number of chemical reactions that give thiol-containing molecules, which play a primary role in cellular redox homeostasis. The FRAP assay is a method of assessing total antioxidant power and is used as an index of the antioxidant potential of the body [13].

The American College of Cardiology/American Heart Association (ACC/AHA) recommended statins regardless of baseline lipid levels in diabetic patients with CVD or who are over the age of 40 years and have one or more CVD risk factors [14]. The major effects of statins are to decrease the number of all atherogenic apolipoprotein B (apo-B) containing lipoprotein particles and raise levels of HDL cholesterol. In addition, statins also possess pleiotropic effects, which include anti-inflammatory and antioxidative properties [15,16].

Various studies have reported a decrease in oxidant and antioxidant markers following treatment with atorvastatin [17,18], simvastatin [19-21], and rosuvastatin [22,23]. However, a review of the literature shows that there are no studies on the effect of statins on the oxidant marker PCC and antioxidant markers FRAP and thiols in patients with DM. Thus, there is a need to further evaluate the effect of statins in patients with T2DM, especially with respect to its effects on OS; one of the fundamental mechanisms underlying atherogenesis.

Among the available statins, rosuvastatin is considered to be a statin of choice in patients at high cardiovascular risk due to its pharmacokinetic efficacy as well as patient safety [24,25]. There is limited data on the antioxidant effect of rosuvastatin in patients with T2DM [22,23]. Data on the antioxidant effects of rosuvastatin in Indian subjects are lacking, thus warranting studies.

Hence, the present study was taken up to assess the effect of rosuvastatin on oxidant and antioxidant markers in patients with T2DM.

MATERIALS AND METHODS

The present prospective interventional study was conducted in the Department of Biochemistry at a tertiary care teaching hospital in South India (Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India) from March 2018 to February 2019. The study was approved by the Institutional Ethics Committee (no. 751/ dated: 02.04.2018) and registered with the Clinical Trials Registry of India (CTRI/2018/07/14771).

Inclusion criteria: Patients diagnosed with T2DM aged between 40 and 70 years as per the revised ADA criteria [4], LDL-C levels over 75 mg/dL [14], and willing to participate were included in the study after obtaining written informed consent. The study included consecutive patients attending the outpatient services of the Department of Endocrinology and Metabolism.

Exclusion criteria: Those having other forms of diabetes, on insulin therapy, on pioglitazone, thyroid disorders not on treatment, those with liver and kidney diseases, other inflammatory diseases, acute illness, malignancy, alcoholics, current smokers, those on lipid-lowering drugs, and those unwilling to participate were excluded from the study.

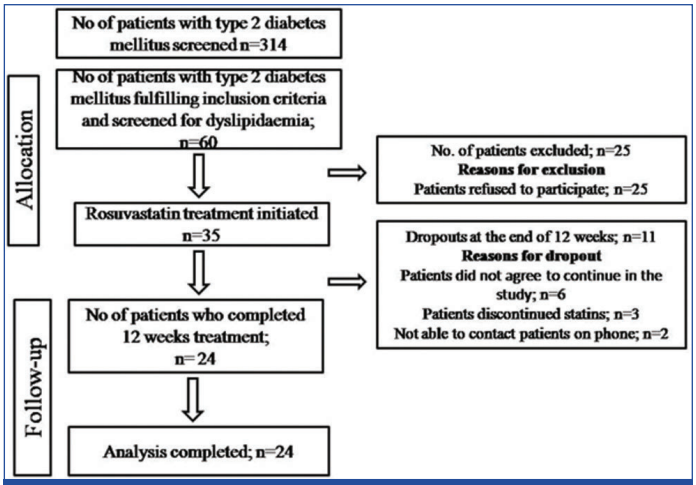
Sample size calculation: Sample size calculation was done based on previous studies [17,22], taking into consideration the pretest and post-test mean and standard deviations, with a power of 80%, an alpha error of 5%, and a two-sided distribution of data. The nMaster software version 2.0 designed by the Department of Biostatistics, Christian Medical College, Vellore, India, was used for this purpose. A sample size of 14 was obtained. However, to strengthen the findings of the study and to account for dropouts, a sample size of 30 was decided for the present study.

Among the 314 cases screened, 60 cases fulfilling the inclusion and exclusion criteria were selected. The reasons for exclusion (n=254) were as follows: age over 70 years (n=33), patients with documented Coronary Artery Disease (CAD) (n=37), patients with Chronic Kidney Disease (CKD) (n=42), alcoholic and/or smokers (n=64), patients on insulin therapy (n=58), and those on statin therapy (n=20). Among these 60 cases, 25 subjects refused to participate after recruitment into the study. The study was initiated in 35 subjects. Among these, six patients did not agree to continue in the study, and five patients dropped out of the study (three patients stopped using statins as they were not satisfied with the statins, while two patients were lost to follow-up). Thus, 24 patients continued in the study to completion [Table/Fig-1].

Study Procedure

Details of medical history and current treatment were collected using a predefined structured proforma. Blood pressure, height, and weight were recorded. Body Mass Index (BMI) was calculated using the formula weight in kg/height in m^2. Waist circumference was measured using a non-elastic tape at a point midway between the lower border of the rib cage and the iliac crest and noted to the nearest half-centimetre. The patient was advised to take one tablet of rosuvastatin 20 mg/day (Med Manor Organics Pvt. Ltd., Uttarakhand, India) orally for a period of 12 weeks [26]. Compliance was verified over the phone.

After 12 hours of overnight fasting, 5 mL of peripheral venous blood samples were collected from all the study subjects at baseline and after 12 weeks of rosuvastatin treatment. One millilitre of blood was



[Table/Fig-1]: Flowchart of the study participants.

transferred into a test tube containing sodium fluoride and potassium oxalate (in a 1:3 ratio of 20 mg/5 mL) anticoagulant and centrifuged at 3000 rotations per minute (rpm) for five minutes to obtain the plasma. Four millilitres were transferred into additive-free tubes, allowed to stand for 30 minutes for clot formation, following which they were centrifuged at 3000 rpm for 15 minutes to obtain serum. Plasma samples were analysed immediately for plasma glucose levels. Serum samples were aliquoted into appropriately labeled vials and stored at -80°C in a deep freezer (Thermo Fisher Scientific, USA) until analysis. The details of the methods and analysers used are shown in [Table/Fig-2] [4,13,27-32].

Parameters	Method	Equipment	Cut-off range
Plasma glucose	Glucose Oxidase Peroxidase (GOD-POD) method using commercial kit from Pathozyme Diagnostics Maharashtra, India	Beckman AU 480 auto analyser (Beckman Coulter, Brea, CA, USA)	Fasting Plasma Glucose: ≥126 mg/dL 2h- PostGlucose Plasma Glucose: ≥200 mg/dL [4]
Serum total cholesterol	Cholesterol oxidase peroxidase method using commercial kit from Agappe diagnostics Ltd., Ernakulum, Kerala, India		<200 mg/dL [27]
Serum triglycerides	Enzymatic colorimetric method using commercial kit from Agappe diagnostics Ltd., Ernakulum, Kerala, India		<150 mg/dL [27]
Serum HDL-C	Selective Inhibition method using commercial kit from Agappe diagnostics Ltd., Ernakulum, Kerala, India	Beckman AU 480 auto analyser (Beckman Coulter, Brea, CA, USA)	<40 mg/dL [27]
VLDL-C	Friedwald formula [28] VLDL-C=TG/5 LDL-C=TC-HDL-C-(TG/5)	Calculated	≤30 mg/dL [27]
Malondialdehyde (MDA)	Measured as thiobarbituric acid reactive substances (TBARS) [29]	Perkin Elmer Lambda 25 UV-Vis Spectrophotometer (Perkin Elmer, Singapore)	0.36-1.24 µmol/L [10]
Protein Carbonyl Content (PCC)	Spectrophotometric method [30]		0.25±0.06 nmol/ mg of protein [31]
FRAP	Spectrophotometric method of Benzie and Strain [13]		0.6-1.6 mmol/L [13]
Protein thiols	Spectrophotometric method [31,32]		182.13±50.54 µmol/L [31]

[Table/Fig-2]: Methods used for biochemical analysis [4,10,13,27-32].

STATISTICAL ANALYSIS

Data distribution was tested using the Kolmogorov-Smirnov test. Data obtained were expressed as mean±standard deviation for normal data and as median (interquartile range) for non normal data. Paired samples t-test/Wilcoxon's signed-rank test was used as appropriate for the comparison of markers at baseline and after 12 weeks of rosuvastatin intervention. The data were transformed to percentages, with the baseline value set at 100%, to nullify the effect of age, BMI, and gender which can affect the baseline oxidant parameters. The changes in markers after follow-up were calculated accordingly with the baseline set at 100%. The association between the markers studied was assessed using linear regression with the GEE, which groups repeated measures for each subject and accounts for correlations that may occur from multiple observations within subjects. All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) Windows version 16.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel spreadsheets (Microsoft, Redmond, WA, USA). A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Among the 314 cases screened, 60 cases fulfilling the inclusion and exclusion criteria were selected. A total of 24 patients continued in the study to completion. The mean age of the study subjects was 48.04±7.96 years. There were 17 male patients (70.8%). The mean BMI was 26.6±2.2 kg/m^2. The mean systolic and diastolic blood pressures were 131.9±8.7 mmHg and 85.6±5.4 mmHg, respectively. Among the study participants, 22 (92%) were on treatment with metformin, 1 (4%) on glycomet, while the remaining 1 (4%) was on ayurvedic treatment at the time of recruitment and was put on metformin later. The treatment of the patients (antidiabetic and statin) was stable during the 12-week follow-up period [Table/Fig-3].

Characteristics	Baseline (n=24)
Age (Years)	48.04±7.96
Males; number (%)	17 (70.8)
Females; number (%)	7 (29.2)
Fasting blood glucose (mg/dL)	159.0±15.4
Body mass index (kg/m^2)	26.6±2.2
SBP (mm Hg)	131.9±8.7
DBP (mm Hg)	85.6±5.4
Medication for diabetes	
1) Metformin	22 (92%)
2) Glycomet	1 (4%)
3) Ayurvedic	1 (4%)
4) Insulin	0%

[Table/Fig-3]: Baseline clinical and biochemical characteristics of the study participants.
Data presented as mean±standard deviation/number (percentage)
BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure

Intervention with rosuvastatin 20 mg/day produced a significant decrease in total cholesterol, TG, LDL-C levels, and a significant increase in HDL-C levels after 12 weeks of follow-up. The percentage reductions were 8.40±3.25% (p<0.001) for TC, 9.13±5.56% (p<0.001) for TG, and 17.0±5.6% (p<0.001) for LDL, while the percentage increase in HDL-C levels was 12.5±6.95% [Table/Fig-4].

In the present study, rosuvastatin 20 mg for 12 weeks produced a beneficial effect on oxidant markers MDA and PCC, as evidenced by a significant decrease in these markers after 12 weeks of intervention compared to baseline. The percentage decrease in MDA was 9.06±3.12% (p<0.001), while that for carbonyl content was 21.2±14.37% (p<0.001).

A significant increase in antioxidant markers FRAP and protein thiols was seen in patients with T2DM after 12 weeks of intervention with rosuvastatin 20 mg compared to baseline [Table/Fig-4]. The percentage increase in FRAP and thiols were 9.08±7.6% (p<0.001) and 11.8±8.4% (p<0.001), respectively [Table/Fig-5].

Parameters	Baseline (n=24)	Follow-up (12 weeks) (n=24)	Percentage change	Direction of change, p-value
Serum TC (mg/dL)	197.67±18.83	181.25±20.50	-8.40±3.25	↓, <0.001
Serum TG (mg/dL)	210.12±31.23	190.17±25.70	-9.13±5.56	↓, <0.001
Serum LDL-C (mg/dL)	108.96±16.24	90.67±16.34	-17.0±5.60	↓, <0.001
Serum VLDL (mg/dL)	42.00±6.19	38.04±5.08	-9.08±5.26	↓, <0.001
Serum HDL-C (mg/dL)	46.70±7.48	52.54±8.74	12.51±6.95	↑, <0.001

[Table/Fig-4]: Percentage changes in lipid profile in patients with T2DM before and after 12 weeks of rosuvastatin treatment.
TC: Total cholesterol; TGL: Triglycerides; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol
Data presented as mean±standard deviation
The data were transformed to percentages with baseline value as 100% before statistical analysis

Parameters	Baseline (n=24)	Follow-up (12 weeks) (n=24)	Percentage change	Direction of change, p-value
Serum MDA (µmol/L)	2.73±0.14	2.48±0.13	-9.06±3.12	↓, <0.001
Serum PCC (ng/mg of protein)	0.26±0.03	0.21±0.05	-21.2±14.37	↓, <0.001
Plasma FRAP (mmol/L)	0.803±0.051	0.873±0.052	9.08±7.60	↑, <0.001
Serum protein thiols (µmol/L)	297.79±23.02	332.95±29.88	11.8±8.39	↑, <0.001

[Table/Fig-5]: Percentage change in markers of Oxidative Stress (OS) in patients with T2DM before and after 12 weeks of rosuvastatin treatment.
MDA: Malondialdehyde; PCC: Protein carbonyl content; FRAP: Ferric reducing ability of plasma
Data presented as mean±standard deviation
The data were transformed to percentages with baseline value as 100% before statistical analysis

To study the association of changes in OS markers with changes in serum lipids, linear regression was performed using the GEE. The model with the best goodness of fit was selected. In patients with diabetes post-intervention, a change in LDL-C was associated with a change in MDA and PCC. Similarly, a change in TG was associated with PCC levels [Table/Fig-6].

Parameters	B±SE*	Wald Chi-square	95% Wald confidence interval		p-value
			Lower	Upper	
Oxidative Stress (OS) marker MDA with lipids					
TC (mg/dL)	0.835±0.1174	50.583	0.605	1.065	<0.001†
LDL-C (mg/dL)	0.453±0.0429	111.549	0.369	0.538	<0.001†
TG (mg/dL)	-0.034±0.0962	0.127	-0.223	0.154	0.722
Oxidative Stress (OS) marker MDA with antioxidant markers					
Protein thiols	-0.149±0.0778	3.683	-0.302	0.003	0.055
FRAP	-0.249±0.0448	30.980	-0.337	-0.161	<0.001†
Oxidative Stress (OS) marker PCC with lipids					
TC (mg/dL)	1.336±0.3639	13.479	0.623	2.049	<0.001†
LDL-C (mg/dL)	0.979±0.2159	20.569	0.556	1.402	<0.001†
TG (mg/dL)	2.042±0.3056	44.641	1.443	2.641	<0.001†
Oxidative Stress (OS) marker PCC with antioxidant markers					
Protein thiols	-0.565±0.2130	7.038	-0.983	-0.148	0.008†
FRAP	-1.453±0.2925	24.677	-2.026	-0.880	<0.001†

[Table/Fig-6]: Association of change in markers of Oxidative Stress (OS) with change in lipids in patients with T2DM.
*B: Beta; SE: Standard error; †statistically significant
TC: Total cholesterol; TG: Triglycerides; LDL-C: Low density lipoprotein-cholesterol;
MDA: Malondialdehyde; FRAP: Ferric reducing ability of plasma; PCC: Protein carbonyl content

All 24 patients were assessed for the safety of rosuvastatin and the development of adverse drug events. None of the patients withdrew from the study because of adverse events known for rosuvastatin, including constipation, diarrhoea, abdominal pain, headache, and nausea. None of the patients had myopathy as assessed by the clinician.

DISCUSSION

In the present study, treatment with rosuvastatin 20 mg for a period of 12 weeks caused a reduction in TC, TG, LDL-C levels, and an increase in HDL-C levels. The lipid-lowering effect of rosuvastatin has been demonstrated in previous studies [33-36]. However, the percentage change reported has been different. This difference can be attributed to the dose used, duration of treatment, and the cause of dyslipidemia and baseline lipid levels in the population studied. A recent meta-analysis concluded that rosuvastatin was the most effective statin in decreasing LDL-C and non-HDL-C [36]. Statins, including rosuvastatin, produce reductions in TC and LDL-C through the inhibition of the Hydroxymethylglutaryl-CoA (HMG-CoA) reductase enzyme and increasing the expression of LDL receptors, thereby increasing the uptake of LDL-C from circulation.

The beneficial effects of statins in CVD risk have been attributed to their lipid as well as non lipid effects. These non-lipid effects are termed as pleiotropic effects, which include the reduction of OS, inflammation, improvement in endothelial function, among others [15,16,37]. Patients with DM have increased OS, which occurs due to an increased generation of ROS as a result of hyperglyacaemia-induced increased production of superoxide production, which, in turn, causes the activation of alternate metabolic pathways, namely increased flux of glucose through the polyol pathway, increased formation of Advanced Glycation End-products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of Protein Kinase C (PKC) isoforms, and overactivity of the hexosamine pathway [38]. OS plays a major role in the development of macrovascular and microvascular complications in patients with diabetes [38].

In line with the observations made in experimental models, rosuvastatin intervention was found to produce a significant decrease in the levels of oxidant markers, MDA (9.06%), and protein carbonyls (21.2%) in the present study. These findings are in agreement with previous studies [17-20]. Villegas-Rivera et al., reported a significant reduction in MDA and PCC after the administration of rosuvastatin 20 mg/day for 16 weeks in patients with diabetic nephropathy [20].

In a study by Manfredini V et al., dyslipidemic T2DM patients not treated with simvastatin had significantly higher plasma MDA and PCC compared to dyslipidemic T2DM patients treated with simvastatin [19]. In an Indian study by Save V et al., atorvastatin was found to significantly decrease MDA levels and the lipoprotein profile in patients with type 2 diabetes [17]. Similar reductions in MDA levels were observed by Usharani P et al., in patients with type 2 diabetes who received atorvastatin in their study [Table/Fig-7] [18].

Rosuvastatin has been shown to attenuate OS in diabetic rats by suppressing Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase, a multisubunit enzyme that catalyses the reduction of molecular oxygen to form the superoxide anion O^{2-}. The superoxide radicals can cause the uncoupling of endothelial Nitric Oxide Synthase (eNOS) and decrease the bioavailability of nitric oxide. These mechanisms have been linked to cardiovascular disorders seen in hypertension, endothelial dysfunction, atherosclerosis, diabetes, etc. Rosuvastatin has been shown to attenuate eNOS uncoupling, increase the bioavailability of NO, and decrease OS in endothelial cells. The findings of the present study thus support these observations of the protective role of rosuvastatin in patients with diabetes [39].

Two antioxidants, FRAP and protein thiols, were studied in the present study. FRAP represents the reducing power of plasma constituents contributed by low molecular weight antioxidants, including antioxidant vitamins C and E, serum bilirubin, and serum UA. Hence, FRAP provides a reliable index of the antioxidant capacity that can be provided by individual antioxidant measurements. Protein thiols represent the antioxidant power of the sulfhydryl groups present on plasma proteins, which act as chain-breaking antioxidants. Albumin is the major thiol-containing protein due to its concentration along with other proteins like cysteine, homocysteine, and GSH [40].

A significant increase in antioxidant markers, FRAP, and protein thiols was seen in patients with T2DM after 12 weeks of rosuvastatin 20 mg treatment compared to baseline [Table/Fig-5]. These findings are in agreement with previous studies in experimental models using rosuvastatin [23] and with other statins in patients with diabetes [19,22]. In an experimental study, Deng J et al., reported that rosuvastatin 10 mg/kg/day for five days decreased oxidative damage as evidenced by an increase in thiols and a decrease in MDA and carbonyl content in diabetic rats [23]. Similarly, Manfredini V et al., showed that simvastatin treatment increased plasma thiol content in dyslipidemic T2DM patients [19]. However, Pereira EC et al., failed to show a beneficial effect of simvastatin on antioxidant status [Table/Fig-7] [17,18,20-22].

An improvement in FRAP and thiols can be supported by the effect of rosuvastatin seen in experimental studies. The antioxidant defence protein heme oxygenase-1 degrades the prooxidant heme and produces carbon monoxide and antioxidant bilirubin [41]. Bilirubin

S. No.	Study	Place and year of the study	Drug used	Dose; duration	Findings for MDA	Findings for FRAP	Findings for PCC	Findings for PT
1.	Save V et al., [17]	Maharashtra, India; 2006	Atorvostatin	10 mg/day; 24 weeks	↓ 5.4% at 6 weeks ↓ 13% at 12 weeks ↓ 18% at 24 weeks	–	–	–
2.	Usharani P et al., [18]	Andhra Pradesh (Present Telangana), India; 2006	Atorvostatin (Placebo controlled)	10 mg/day; 8 weeks	↓ Pretreatment: 3.46±0.5 nmol/mL Post-treatment: 2.16±0.17 nmol/mL	–	–	–
3.	Villegas-Rivera G et al., [20]	Mexico; 2015	Eze/SIMV Rosuvastatin	10/20 mg/day; 16 weeks 20 mg/day; 16 weeks	↓ (Baseline 0.92±0.20 µM; Follow-up 0.52±0.10 µM) ↓ (Baseline 0.82±0.15 µM; Follow-up 0.53±0.10 µM)	–	–	–
4.	Pereira EC et al., [21]	Brazil; 2004	Simvastatin	20 mg/day; 2 months				No change (data presented as graphs)
5.	Koksal M et al., [22]	Turkey; 2011	Atorvastatin Rosuvastatin	20 mg/day; 3 months 10 mg/day; 3 months	NC; -6.54±22.3% NC; 0.76±20.32%	↑; 17.69±33.89% ↑; 16.00±36.89%		
6.	Present study	Andhra Pradesh, India; 2024	Rosuvastatin	20 mg/day; 12 weeks	↓; 9.06%	↓; 21.2%	↑; 9.08%	↑; 11.8%

[Table/Fig-7]: Comparison of results with previous reports [17,18,20-22].
FRAP: Ferric reducing ability of plasma; MDA: Malondialdehyde; PCC: Protein carbonyl content; PT: Protein thiols; NC: No change; ↓=Decrease; ↑=Increase

has potent antioxidant power and is a component of the FRAP. Rosuvastatin has been shown to upregulate heme oxygenase-1 and thus increases its metabolite bilirubin. Addition of exogenous bilirubin was shown to completely abolish NADPH oxidase-dependent ROS production [42]. The improvement in protein thiols is supported by the observation of rosuvastatin-induced increased expression of thiol group-containing enzymes, i.e., GSH synthase, GPx, GR, and the enzymes involved in GSH synthesis [43].

Thus, the pleiotropic effects of rosuvastatin observed in experimental models are supported by the findings of the present study. Furthermore, these effects are associated with the lipid-lowering effect of rosuvastatin. Changes in lipids showed an association with oxidative markers [Table/Fig-6], showing that a decrease in lipids, which form the major substrates for oxidant injury, causes a decrease in oxidant markers. Long-term prospective clinical trials are required to establish the role of the pleiotropic effects in decreasing CV endpoints in patients with T2DM.

Limitation(s)

The major limitation in the present study was the lack of a control group. Additionally, advanced lipid parameters were not assessed in the present study.

CONCLUSION(S)

The findings of the present study suggest that high-intensity statin, i.e., rosuvastatin 20 mg for 12 weeks, produces a beneficial effect on CV risk in patients with T2DM through its lipid and non-lipid effects. Rosuvastatin showed a lipid-lowering effect (↓TC, TG, LDL), an increase in the antioxidant and anti-atherogenic HDL, and decreasing OS by causing a decrease in lipid and protein oxidation markers and an increase in antioxidants. The decrease in OS and the LDL-C levels can thus decrease the formation of oxidised LDL, which initiates the atherosclerotic process. The findings need to be further strengthened in a large trial.

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