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Original Article

Elevated oxidative stress among coronary artery disease patients on statin therapy: A cross sectional study

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ABSTRACT

Background: Statins are a major group of drugs that reduces LDL-C levels, which are proven to have other beneficial effects such as preventing coronary events. The objective of this study was to evaluate oxidative stress and select novel coronary artery disease risk factors among coronary artery disease patients on statins.

Methods: In this observational, cross-sectional study, we compared total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, apolipoprotein B, lipoprotein (a), homocysteine, reduced glutathione, glutathione peroxidase, superoxide dismutase, ascorbic acid, malondialdehyde and oxidized LDL among male coronary artery disease patients on statin therapy (group 2, $n = 151$) with sex-matched, diabetic patients (group 3, $n = 80$) as well as healthy controls (group 1, $n = 84$).

Results: Total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were significantly lower among subjects of group 2 compared to other two groups. The novel risk factors studied did not differ significantly between groups, except for a higher homocysteine level among group 2 subjects compared to the other two groups. Elevated oxidative stress, indicated by lower reduced glutathione, glutathione peroxidase, and ascorbic acid as well as higher malondialdehyde and oxidized LDL was observed among group 2 subjects. Triglycerides, HDL-cholesterol, ascorbic acid and malondialdehyde were found to be independent predictors for coronary artery disease among this study population.

Conclusions: Though coronary artery disease subjects had healthy lipid profile, oxidative stress, a recognized risk factor for coronary events, was still elevated among this patient group. Novel risk factors were not found to be major predictors for coronary artery disease among the study subjects.

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1. Introduction

National Cholesterol Education Program Adult Panel III guidelines identify the reduction of low density-lipoprotein cholesterol [LDL-C] as the primary target of therapy for coronary artery disease [CAD] patients.[1] Statins are a major group of drugs that reduces LDL-C levels by inhibiting HMG-CoA reductase, the rate limiting enzyme involved in the hepatic synthesis of cholesterol. Since the observed advantage of statin therapy in reducing adverse cardiovascular events is larger than that expected on the basis of lipid lowering alone, statins are attributed to have cholesterol-independent beneficial effects also.

Though LDL-C remains an important therapeutic target among CAD patients, factors such as apolipoprotein B [apo B], lipoprotein (a) [Lp(a)], homocysteine [Hcy], oxidative stress etc. have also been identified as contributory factors for CAD and have recently emerged as secondary targets of therapy.[2–4] Apo B represents the circulating LDL particle number, and it has been recognized that CAD risk is more directly related to the number than to the cholesterol content of lipoproteins.[5] Lp(a) is a LDL like particle to which a glycoprotein, apolipoprotein(a) is covalently linked and it has higher pro-atherogenic, pro-thrombotic potential compared with LDL.[6] Elevated Hcy concentration induces oxidative stress and damages endothelium and is considered another important CAD risk factor.[7] Oxidative stress contributes to the initiation and exacerbation of several chronic diseases including atherosclerosis. Elevated oxidative stress has been reported among CAD patients in earlier studies.[8] Previous investigations have shown that statins can markedly reduce circulating apo B concentrations as well as oxidative stress.[9,10] However, the effect of statins on Lp(a) and Hcy has not been fully established.[11,12]

Novel risk factors and level of oxidative stress among CAD patients of Kerala on statin therapy is not thoroughly investigated. A community-based study in Kerala has shown that this population has high prevalence of conventional risk factors for non-communicable diseases.[13] A recent study reported higher oxidative stress among CAD subjects of this population,[14] still there is paucity of data in this direction. Hence this observational, cross sectional study was undertaken to find out if CAD patients on statins (a class of drugs with known pleiotropic effects) had lipid profile, apo B, Lp(a), Hcy and the degree of oxidative stress comparable to healthy controls.

2. Materials and methods

This study was conducted for a period of 16 months at a tertiary care university hospital in Kerala in accordance with the Declaration of Helsinki. The protocol for the study was approved by the Institutional Ethics Committee, and informed consent was obtained from all the participating subjects.

2.1. *Subjects*

Men aged 35–70 years, who presented to Cardiology, Endocrinology and Comprehensive Health Care out-patient departments [OPD] of the hospital for routine health check-up, satisfying the inclusion criteria and willing to participate in the study, were recruited. We screened 477 patients in these OPDs and 315 were recruited in the following groups. 84 subjects with normal blood glucose level, normal blood pressure, without thyroid abnormalities, without prior history of angina or myocardial infarction, a normal 12 lead ECG and absence of inducible ischemia on stress test were included in group 1 (control group). 151 subjects with previously documented myocardial infarction or inducible ischemia on stress test or angiographically proven CAD and under statin therapy were included in group 2. Group 3 included 80 patients with previously diagnosed type 2 diabetes mellitus, but without CAD, thyroid or renal complications. Type 2 diabetes was diagnosed based on WHO diagnostic criteria for diabetes [fasting blood glucose levels >126 mg/dL].[15] Subjects of groups 1 and 3 were not on statins previously. Subjects with positive history of anti-hypertensive medications at the time of recruitment were considered to be hypertensives. A current tobacco user is defined in this study as someone smoking cigarettes/beedis or using smokeless tobacco within past 3 months of recruitment. The subjects furnished details regarding their diet and life style habits in a dietary questionnaire provided during their recruitment. Details regarding medication of the enrolled subjects were procured from the patient file.

2.2. *Laboratory analysis*

2 ml of fasting blood in EDTA and 2 ml without anticoagulants was collected from each subject. The samples were centrifuged immediately and serum/plasma was separated.

2.2.1. *Oxidative stress parameters*

Parameters analyzed to evaluate oxidative stress include reduced glutathione [GSH], glutathione peroxidase [GPx], superoxide dismutase [SOD], ascorbic acid, malondialdehyde [MDA] and oxidized LDL [oxLDL]. Erythrocytes were washed thrice with ice-cold physiological saline, lysed with de-ionized water and used for estimation of GSH, GPx and MDA. GSH was estimated using the method of Beutler et al.[16] GPx was assayed according to Paglia and Valentine[17] and as modified by Lawrence and Burk.[18] MDA was estimated by the method of Jain et al.[19]

SOD was assayed in serum based on the method of Marklund and Marklund[20] and as modified by Nandi and Chatterjee.[21] Ascorbic acid was estimated in plasma by dinitrophenyl hydrazine assay.[22] OxLDL was estimated in plasma using Mercodia Oxidized LDL ELISA kit (Mercodia, Sweden) based on direct sandwich ELISA technique.

2.2.2. *Novel CAD risk factors*

Apo B was analyzed in serum by immunoturbidimetry using kits from Daiichi Chemical Co., Japan. Lp(a) was determined in serum by ELISA using ELITEST-Lp(a) kit from Hyphen Biomed, France. Hcy concentrations were assayed in plasma samples by ELISA using kits from Bio-Rad, USA.

2.2.3. *Lipid parameters*

Lipid parameters assayed included total cholesterol [TC], triglycerides [TG], HDL cholesterol [HDL-C], and LDL-C, which

were measured in serum using kits from Roche in Hitachi 912 auto-analyzer.

2.3. Statistical analysis

Categorical variables were represented as numbers and/or percentages. All the other parameters were represented as mean value and standard deviation. ANOVA was used to compare mean values of the parameters between different groups and a p value <0.05 was considered to be statistically significant. If test of homogeneity of variances was significant, *post hoc* analysis was done by Games–Howell test. If test of homogeneity of variances was not significant, *post hoc* analysis was carried out by Tukey's test. A p value <0.05 was considered statistically significant in *post hoc* analysis also.

Pearson's correlation analysis was performed to analyze the degree of correlation between different parameters in each group. Further, the groups were considered as a single sample and after adjusting for age, multivariate linear regression was performed to identify independent predictors of CAD in this population. Statistical analysis of data was done using IBM SPSS software, version 21.0.

3. Results

The number of current tobacco users was 13, 17 and 11 respectively among groups 1, 2, and 3. Subjects with hypertension were 91 among group 2 and 33 among group 3 subjects. Among group 2 patients, 78 subjects were diabetic. All the CAD subjects were on statin therapy and the diabetic subjects were on oral hypoglycemic agents or insulin therapy. Patients with hypertension in groups 2 and 3 were on anti-hypertensive drugs. The clinical profile of the subjects is given in Table 1.

3.1. Oxidative stress parameters

Results of analysis of parameters to measure oxidative stress are given in Table 2. GSH, GPx, ascorbic acid, MDA (all $p = 0.000$) and oxLDL ($p = 0.001$) had significant p values, whereas SOD did not have a significant p value ($p = 0.664$) between the groups upon preliminary data analysis. On *post hoc* analysis, it was found that controls had significantly higher GSH compared to both CAD group and diabetics ($p = 0.000$ for both). GPx was lower for CAD group compared to controls and diabetics ($p = 0.000$ and 0.001 respectively). GPx was significantly lower for diabetic patients also compared to controls ($p = 0.000$). Ascorbic acid was lower among CAD group compared to controls and diabetics ($p = 0.000$ for both). MDA was higher among CAD subjects compared to controls and diabetics ($p = 0.000$ for both). CAD subjects had significantly higher oxLDL levels also compared to controls and diabetics ($p = 0.008$ and 0.007 respectively).

No correlation was observed between the oxidative stress parameters, nor between these and lipid parameters or novel risk factors upon analysis. Upon regression analysis, GPx ($B = -0.027$, $p = 0.000$), ascorbic acid ($B = -0.043$, $p = 0.000$), and MDA ($B = 0.143$, $p = 0.000$) were found to be major predictors for CAD.

Table 1 – Clinical characteristics of subjects.

	Group 1 $n = 84$	Group 2 $n = 151$	Group 3 $n = 80$
Variables			
Mean age (years)	50.1	56.7	52.4
Smokers (n)	13 (15%)	17 (11%)	11 (13%)
Hypertensives (n)	– (0%)	91 (60%)	33 (41%)
Diabetics (n)	– (0%)	78 (51%)	80 (100%)
FBS in mg/dL (Mean ± SD)	97 ± 13	116 ± 27	131 ± 21

FBS: fasting blood sugar, mg/dL: milligram/deciliter, SD: standard deviation.

3.2. Novel CAD risk factors

The results of biochemical analysis of apo B, Lp(a) and Hcy are given in Table 3. Though apo B was higher among controls and diabetics compared to CAD group, the p value was not significant between the groups ($p = 0.21$). Mean Lp(a) concentrations were not significantly different between the groups ($p = 0.094$). Mean Hcy values of the three groups had significant p value ($p = 0.007$) on preliminary analysis of data and post hoc analysis showed that it was significantly higher for CAD subjects compared to controls and diabetics ($p = 0.008$ and 0.03 respectively), while it did not show any significant difference between control and diabetic groups ($p = 0.837$).

Apo B had moderate positive correlation with TC and LDL-C among controls ($r = 0.414$ and 0.437 respectively) as well as CAD subjects (0.315 and 0.441 respectively), whereas no such correlation was observed among diabetic subjects. Novel risk factors were not found to be major predictors for CAD on regression analysis.

3.3. Lipid parameters

The results of biochemical analysis of lipid parameters are given in Table 3. Upon preliminary analysis it was found that TC, TG, HDL-C and LDL-C had significant p values ($p = 0.000$). *Post hoc* analysis showed that TC was significantly higher for controls and diabetics compared to CAD subjects ($p = 0.000$ for both). Control group had elevated TC levels compared to diabetics also ($p = 0.001$). TG was significantly higher for controls compared to CAD subjects ($p = 0.001$), whereas it was significantly higher for diabetic subjects compared to CAD patients ($p = 0.000$) as well as controls ($p = 0.05$). HDL-C showed significant reduction among CAD subjects and diabetics compared to controls ($p = 0.000$ and 0.025 respectively), but was not found to be significantly different between CAD and diabetic patients ($p = 0.252$). Mean LDL-C was found to be significantly lowered among CAD subjects compared to subjects of groups 1 and 3 ($p = 0.000$ for both).

TC was positively correlated with LDL-C among group 1, 2 and 3 ($r = 0.885$, 0.660 and 0.734 respectively). TG had weak positive correlation with TC and LDL-C ($r = 0.387$ and 0.304 respectively) and a weak negative correlation with HDL-C ($r = -0.334$). Among lipid parameters, TG ($B = -0.001$, $p = 0.002$) and HDL-C ($B = -0.006$, $p = 0.003$) were found to be major predictors for CAD upon regression analysis.

Table 2 – Results of analyses of oxidative stress parameters.

Parameters (Mean ± SD)	Group 1	Group 2	Group 3	*p* Value and CI (groups 1 & 2)	*p* Value and CI (groups 1 & 3)	*p* Value and CI (groups 2 & 3)
GSH (nmoles/g Hb)	7 ± 0.7	5.3 ± 0.9	5.3 ± 0.8	0.000 (1.44 to 2.03)	0.000 (1.35 to 2.02)	0.926 (−0.33 to 0.24)
GPx (IU/g Hb)	18.6 ± 6.3	14.7 ± 5.9	15.9 ± 6	0.000 (3.13 to 4.82)	0.000 (1.74 to 3.68)	0.001 (−2.11 to −0.43)
SOD (U/mL serum)	5 ± 1	4.9 ± 0.9	4.8 ± 1.1	0.703 (−0.22 to 0.46)	0.701 (−0.26 to 0.53)	0.991 (0.33 to 0.36)
Ascorbic acid (mg/L)	10.7 ± 2.4	8.8 ± 1.8	10.1 ± 2.3	0.000 (1.37 to 2.66)	0.072 (0.05 to 1.58)	0.000 (−1.96 to −0.55)
MDA (nmoles/g Hb)	14.1 ± 2.1	17.2 ± 1	14.5 ± 0.6	0.000 (−3.71 to −2.55)	0.303 (−0.92 to 0.21)	0.000 (2.53 to 3.02)
oxLDL (U/L)	64 ± 24.5	76.5 ± 30.2	63.3 ± 26.3	0.008 (−22.37 to −2.69)	0.837 (−11.19 to 12.54)	0.007 (3.09 to 23.33)

SD: standard deviation, CI: Confidence Interval for mean difference, nmoles/g Hb: nanomoles per gram hemoglobin, IU/g Hb: international units per gram hemoglobin, U/mL: units per milliliter, mg/L: milligram/liter, U/L: units per liter.

4. Discussion

The detrimental effects of higher oxidative stress on atherosclerosis include endothelial dysfunction, lipid peroxidation, activation of several inflammatory pathways etc.[23] It has been demonstrated earlier that higher oxidative stress is common among subjects with CAD.[24] Several clinical studies suggest that statins can reduce oxidative stress among CAD patients.[10,25] But it was seen in our study that subjects under statin therapy had lower antioxidant status as indicated by low GSH, GPx and ascorbic acid compared to non-statin groups, viz. diabetics as well as healthy controls. High rate of lipid peroxidation and oxidation of LDL was also noted among our CAD patients. This is incongruous with studies that show that oxidative stress among CAD subjects was reduced and was comparable to healthy individuals after undergoing statin therapy.[26] But there are few studies available that have shown that CAD subjects have higher oxidative stress compared to healthy controls even while being clinically stable and under statin therapy.[27] The level of oxidative stress among diabetic subjects was lower than those in CAD group, but was higher compared to healthy controls. It has been proven earlier that higher oxidative stress is prevalent among diabetes patients[28] and plays a critical role in the development of both microvascular and cardiovascular complications of diabetes.[29]

Studies have shown that statins can markedly reduce circulating apo B levels, thereby reducing their CAD risk.[30] But in our study the apo B levels of CAD patients under statin therapy was not significantly lower compared to non-statin groups. The results of The MERCURY II trial show that to attain clinically relevant reduction in apo B levels (<90 mg/dL), the LDL-C concentration should be reduced below 70 mg/dL among hypertriglyceridemics and below 80 mg/dL among non-hypertriglyceridemics.[31] The elevated apo B concentrations among CAD subjects of our study could be because their LDL-C level was higher than the measure required for a significant apo B reduction. Since apo B levels were correlated with LDL-C levels in our study subjects, further reduction in LDL-C by aggressive statin therapy may concurrently reduce their apo B levels also.

Hitherto, the studies on the effect of statins on Lp(a) are sparse and the data generated from these are disparate.[32,33] The results from the present study show that Lp(a) concentration did not differ significantly between CAD patients on statin therapy compared to both non-statin groups. An earlier study among CAD patients of Kerala has shown that their Lp(a) level was not considerably high when compared to healthy controls.[34] Most of the available literature show that statin therapy has no detectable or clinically relevant effect on Hcy levels.[35,36] Hcy concentrations of the CAD patients of this study was significantly elevated than diabetics and controls and this level is higher than that reported by a study in related population.[37]

The CAD patients of this study had healthier lipid profile, as expected, in terms of TC, TG and LDL-C level compared to the subjects of the other two groups. Elevated LDL-C noted among controls and diabetics of this study was comparable to earlier reports from this population.[38] The mean HDL-C level among the CAD subjects of this study was low, and is consistent with results from studies on other Indian CAD populations.[39,40] An earlier study has shown that HDL-C is low even among general population of this region.[13] It is noteworthy that dyslipidemia (TG > 150 mg/dL and HDL-C <40 mg/dL), an established risk aggravator for CAD,[41] was

Table 3 – Results of analyses of lipid parameters and novel CAD risk factors.

Parameters (Mean ± SD)	Group 1	Group 2	Group 3	*p* Value and CI (groups 1 & 2)	*p* Value and CI (groups 1 & 3)	*p* Value and CI (groups 2 & 3)
Total cholesterol (mg/dL)	208.7 ± 40	147.7 ± 32.3	182.5 ± 49.6	0.000 (48.9 to 73.1)	0.001 (9.6 to 42.9)	0.000 (20.3 to 49.3)
Triglycerides (mg/dL)	138.7 ± 49.8	114.6 ± 46.3	156 ± 46.1	0.001 (8.9 to 39.3)	0.05 (−34.8 to 0.99)	0.000 (−57 to −25.9)
HDL-cholesterol (mg/dL)	47.5 ± 10.6	39.5 ± 14.7	42.3 ± 10	0.000 (3.94 to 12.1)	0.025 (0.53 to 9.86)	0.252 (−6.96 to 1.35)
LDL-cholesterol (mg/dL)	129.8 ± 32.9	91.7 ± 27.4	121.7 ± 45.1	0.000 (28.03 to 48.1)	0.399 (−6.69 to 22.87)	0.000 (−43.24 to −16.71)
Apo B (mg/dL)	96.3 ± 17.2	91.2 ± 19.9	93.1 ± 18.8	0.185 (−1.69 to 11.68)	0.589 (−4.49 to 10.91)	0.802 (−8.44 to 4.87)
Lp(a) (mg/dL)	18.8 ± 7.6	21.8 ± 9.4	21.1 ± 8.4	0.077 (−6.27 to 0.25)	0.328 (−6.03 to 1.48)	0.852 (−2.48 to 3.96)
Hcy (μmoles/L)	13.6 ± 5.6	16.6 ± 8.6	14.1 ± 5.1	0.008 (−5.33 to −0.65)	0.837 (−2.67 to 1.63)	0.03 (0.19 to 4.75)

SD: standard deviation, CI: Confidence Interval for mean difference, μmoles/L: micromoles/liter, mg/dL: milligram/deciliter.

more prevalent among diabetic patients in this study (29%) compared to controls (9%) or CAD patients (16%).

5. Conclusions

In spite of therapeutical intervention by statins, a drug with proven antioxidant capabilities, CAD subjects in this study had higher oxidative stress. Lipid profile levels of CAD patients were healthier compared to diabetics or controls. Novel risk factors such as apo B, Lp(a) etc. were not considerably different between these three groups.

6. Limitations of the study

The type/dosage/duration of statin treatment was not considered while recruiting the subjects. The parameters considered were not measured before statin therapy among CAD subjects. Differences in micronutrient intake in the form of vitamin supplements and dietary differences (both inter-group and intra-group) must also have influenced the results.

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Conflicts of interest

All authors have none to declare.

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