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EFFECT OF ATORVASTATIN ON ADVANCED OXIDATION PROTEIN PRODUCTS AND MALONDIALDEHYDE IN DYSLIPIDEMIC PATIENTS WITH AND WITHOUT DIABETES.

***BENEDICTA D'SOUZA, MICHELE P. CHRISTIAN, RAVIRAJA, ASHOK PRABHU, BEENA V. SHETTY AND VIVIAN D'SOUZA**

Manipal University, Kasturba Medical College, Centre for Basic Sciences, Bejai, Mangalore 575004.

ABSTRACT

The aim of this study was to ascertain the effect of atorvastatin on oxidative stress parameters in dyslipidemic patients with or without diabetes. Advanced oxidation of protein products(AOPP), was measured as a marker for protein oxidation, malondialdehyde was studied as a marker of lipid peroxidation and glutathione (G-SH) was estimated to study the antioxidant potential of these patients. The results were compared with normal healthy control subjects. This study suggests that there is a significant increase in both AOPP and MDA in dyslipidemic, diabetic patients with or without atorvastatin. There is no significant change in antioxidant G-SH levels in these patients, compared to the control subjects. This study indicates that atorvastatin increases the oxidative stress.

KEY WORDS : AOPP, MDA, G-SH, Atorvastatin, Dyslipidemic patients.

BENEDICTA D'SOUZA
Manipal University, Kasturba Medical College,
Centre for Basic Sciences,Bejai, Mangalore 575004.

INTRODUCTION

Reactive oxygen species(ROS) are involved in the pathogenesis of several diseases. ROS can react with DNA, lipids, proteins, carbohydrates and damage these macromolecules. Proteins are the main targets of oxidation in plasma. Oxidative modifications of proteins are good oxidative stress markers with much better stability than lipids (1,2). Advanced oxidation protein products(AOPP), formed by the action of free radicals on proteins may act as mediators of inflammatory activity and increases the oxidative stress(3). Several forms of protein oxidation can occur, including the formation of cross linking molecules by oxidation of sulfhydryl groups. Polyunsaturated fatty acids are also highly susceptible to free radical attack.Malondialdehyde (MDA) , formed by the lipid peroxidation, is a good indicator of oxidative stress(4). Diabetes is associated with increased modification of proteins. Accumulation of AOPPs has been linked to chronic inflammation and monocyte activation which suggests that AOPPs might be potential inducers of cellular inflammation(5). Endothelial dysfunction is frequently found in diabetic patients. Atorvastatin belongs to the class of cholesterol reducing drugs. This study was conducted to assess the effect of atorvastatin on selected oxidative stress parameters ie. lipid peroxidation by product MDA, protein oxidation product AOPP and antioxidant parameter like glutathione(GSH), in dyslipidemic patients with and without diabetes.

MATERIALS AND METHODS

The study group consist of 110 subjects between the age group of 35 to 65 years of both sexes. Patients were attending OPD in Kasturba medical college hospitals, Mangalore. Informed consent was taken from all the subjects. They were divided into 5 groups.Group 1 (n=20): is healthy control subjects.Group 2 (n=30): newly diagnosed dyslipidemic , diabetic patients.Group 3 (n=20) :dyslipidemic, type 2 diabetic patients , received atorvastatin 10mg daily at night. Group 4 (n=28) :dyslipidemic, nondiabetic patients, received no drug and Group 5 (n=12) : dyslipidemic nondiabetic patients received atorvastatin 10 mg once daily at night. Exclusion criteria : Patients with cardiac disease, cancer patients, acute and chronic infection with nephrotic syndrome, renal failure and hemodialysis patients, liver disease, allergic reactions, smokers and alcohol abusers are excluded from the study. Sample collection: 5ml of venous blood was collected in EDTA bottles from dyslipidemic patients and normal healthy individual. 0.2ml of whole blood was used for Glutathione(G-SH) estimation. Blood samples were centrifuged at 3000rpm for 10 minutes. Cells were washed with 0.9% normal saline and used for malondialdehyde(MDA) estimation. MDA was measured by modified method of Stocks and Dormandy (6). AOPP was measured by the method of Witko-Sarsat et al(7). G-SH was estimated by Ernest Beutler's method (8).

Statistical analysis was done by ANOVA, F test.

RESULTS

AOPP levels were greatly increased in all the dyslipidemic patients with and without diabetes, compared to control subjects. But increase in AOPP was statistically significant($p < 0.025$) in dyslipidemic, nondiabetic patients with atorvastatin drug(Table 1) . Among the other groups there is no significant change in AOPP levels. MDA levels were significantly($p < 0.001$) increased in all the dyslipidemic patients with and without diabetes and on atorvastatin, compared to the control subjects(Table 2) . Among the other groups also there is a significant increase in MDA levels ($p<0.001$).

There is no change in G-SH levels in dyslipidemic patients with and without diabetes and on atorvastatin therapy, compared to the control group (Table3). Among the other groups, GSH is decreased in dyslipidemic

nondiabetic patients with atorvastatin therapy, but the decrease in GSH is not statistically significant.

DISCUSSION

Oxidative stress is tissue injury resulting from a disturbance in the equilibrium between the production of ROS and antioxidant defence mechanisms. The antioxidant defences are able to protect against the deleterious effects of ROS. But whenever there is an increase in oxidant generation and decrease in antioxidant protection, accumulation of ROS takes place resulting in tissue damage(9,10). In the present study, we have found an increase in AOPP and MDA , which may cause excessive oxidative stress in dyslipidemic patients. There is no significant change in antioxidant GSH levels in dyslipidemic patients compared to the control subjects. The slight decrease in GSH in dyslipidemic nondiabetic patients with atorvastatin therapy, may be due to its increased utilization to scavenge the ROS(11, 12). Atorvastatin increases the oxidative stress in dyslipidemic patients. Diabetes is associated with increased modification of proteins and accumulation of advanced glycation end products. Accumulation of AOPP may induce cellular inflammation and monocyte activation. Diabetes is also associated with dyslipidemia and increased oxidative stress(13,14). Statins are useful in diabetic patients due to their low density lipoprotein cholesterol(LDL-C) lowering effects. Koksal M et al have reported that atorvastatin reduces oxidative stress in diabetic patients with hyperlipidemia(15). Marcello Arca et al have shown in their study that, atorvastatin therapy decreases oxidative stress in patients with familial combined hyperlipidemia(16). According to Eugenio Barone et al, long term high dose atorvastatin decreases brain oxidative stress in Alzhiemer disease (17). A surprising finding in the present study was that AOPP and MDA levels, which are the markers of oxidative stress, are significantly increased in dyslipidemic patients with or without diabetes and on atorvastatin therapy. Increased oxidative stress may be due to reduction in antioxidants. Additional studies are required to study the activity of antioxidant enzymes and to ascertain the role of atorvastatin on oxidative stress.

TABLE 1

AOPP (µmol/L) levels in dyslipidemic patients

Groups		N	Mean	S.D.
1.	Control	20	0.1470	0.06364
2.	Dyslipidemic,diabetic without atorvastatin	30	0.2585	0.17571
3.	Dyslipidemic, nondiabetic, without atorvastatin	28	0.2192	± 0.08739
4.	Dyslipidemic, diabetic, with atorvastatin	20	0.2599	± 0.27127
5.	Dyslipidemic, nondiabetic, with atorvastatin	12	0.3478*	± 0.21720

ANOVA, F = 2.904, *p = 0.025 sig (significant compared to control group), S.D.= Standard deviation

TABLE 2

MDA (nmol/dL)levels indyslipidemic patients

Groups		N	Mean	S.D.
1.	Control	20	40.650	± 14.992
2.	Dyslipidemic,diabetic without atorvastatin	30	52.20*	± 11.146
3.	Dyslipidemic, nondiabetic, without atorvastatin	28	43.535*	± 13.595
1.	Dyslipidemic, diabetic, with atorvastatin	20	52.60*	± 11.807
2.	Dyslipidemic, nondiabetic, with atorvastatin	12	54.08*	± 8.218

ANOVA, F= 4.894, * p < 0.001 vhs(very highly significant compared to control group), S.D.= Standard deviation

TABLE 3
G-SH (mg/dL) levels in Dyslipidemic patients

Groups		N	Mean	S.D.
4.	Control	20	11.617	± 8.3199
5.	Dyslipidemic,diabetic without atorvastatin	30	9.3147	±6.0038
6.	Dyslipidemic, nondiabetic, without atorvastatin	28	11.684	±7.3700
3.	Dyslipidemic, diabetic, with atorvastatin	20	11.536	±7.7702
4.	Dyslipidemic, nondiabetic, with atorvastatin	12	8.1742	±2.7514

ANOVA, F= 0.993, p=0.415 NS (Not significant)

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