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Plasma oxysterol level in patients with coronary artery stenosis and its changes in response to the treatment with atorvastatin

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Abstract

Background: Considering the increasing incidence of coronary artery stenosis and its related complications, the importance of its etiology and inconsistent reports we aimed to determine the relationship between oxysterol, serum levels and severity of coronary atherosclerosis and effect of statins on oxysterol.

Methods: A total of 85 patients referred to Taleghani Hospital, Tehran, Iran during 2011-2012 with coronary artery stenosis more than 75%, as determined by angiography, participated in the current study. Their demographic information and history of smoking and taking atorvastatin was carefully recorded. Two milliliters of venous blood was obtained from each patient. The serum oxysterol level of samples was measured using the enzyme-linked immunosorbent assay (ELISA) method. Statistical analysis was performed using SPSS v.19.

Results: Eighty five patients completed the study. Mean age of patients was 64.4 years; 51 (60%) were male; 55 (68%) had acute coronary syndrome and 30 (32%) had chronic stable angina. Mean±SD of plasma level of oxysterol was 24.8±0.2 pmol/ml. The normal range of oxysterol level was 13pmol/ml. Mean±SD of plasma oxysterol level in patients under statin therapy was 24.4±2.1 pmol/ml. In patients without receiving statins, plasma oxysterol level was 26.38±1.6pmol/ml.

Conclusion: Findings of the present study indicated significant correlation between serum oxysterol and severity of coronary artery stenosis. It also demonstrated that receiving atorvastatin is associated with significant reduction of plasma oxysterol level.

Keywords: Coronary stenosis, Atorvastatin, Hydroxymethylglutaryl-CoA Reductase Inhibitors, Anticholesteremic agents.

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Introduction

Nowadays, cardiovascular diseases are amongst the major causes of mortality and morbidity worldwide. In Iran, like other parts of the world, studies demonstrate that death toll related to cardiovascular complications is high (1). According to the reports by the Iranian Ministry of Health 39.3% of the deaths in 2005 were due to cardiovascular diseases. Atherosclerosis, a multifactorial disorder, is one of the major causes of

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* *We are very sorry to announce that the author passed away.*

stroke and myocardial infarction (2). Several causes have been described to be associated with the increased risk of acquiring atherosclerosis. These include high fat diets, hypertension, genetic risk factors, and using some medications such as anticonvulsants (3-6). It has long been believed that cholesterol level is responsible for developing atherosclerosis, but currently researchers identified oxidized fats to be major contributors of atherogenesis (7). Plasma oxysterols are indicator of lipid oxidative stress and major oxidized bioactive lipids, produced during low density lipoprotein (LDL) oxidation. Their coalescence in blood vessels causes cytotoxicity, apoptosis, monocyte differentiation and formation of cell foam and consequently damage and malfunction of vascular endothelium (8, 9). They appear to be implicated in the activation of mitochondrial pathway of apoptosis and inflammatory and immunological pathways (10-12). Elevation of the plasma level of oxysterols has been observed in the patients with atherosclerosis and directly correlates with the advancement of atherosclerosis in affected areas (9, 13). In 2009 Poli et al. (14) defined six steps for the atherosclerotic effects of oxysterols: 1) endothelial cell malfunction, 2) increase of cellular adherence and secretion of chemicals which attract the monocytes to the endothelium, 3) Production of foam cells, 4) Overproduction of extracellular matrix components and increasing the interaction between cells and macrophages, 5) Inflammation and formation of fibrotic cup and 6) Cellular apoptosis. Studies by Liu et al. and Rosklint et al. demonstrated that 25-hydroxycholesterol induce the production of interleukin-18 and interleukin-1β by macrophages and contribute to the inflammation and sclerogenesis in the arteries (15, 16).

In 2007, Arca et al. showed that oxysterols are present in hyperlipidemic patients in high levels (17). In 2008, Szuchman et al. demonstrated that oxysterols are highly elevated in the serum of patients with diabetes and hypercholesterolemia when compared to control groups (18).

It appears that oxysterol and its isomers are important atherogenic risk factors due to their high cytotoxicity. In the current research we studied the level of plasma oxysterols in patients with severe coronary artery stenosis and the effects of treatment with atorvastatin.

Methods

Patients

Fifty eight patients referred to the Taleghani Hospital were studied in this cross-sectional study. Patients included those suffering from stable angina or coronary artery stenosis, as determined by angiography and were under treatment. Patients with coronary artery stenosis were referred to the emergency ward and admitted to the CCU for acute coronary syndrome; this group was also examined by angiography. Patients who agreed to participate in the study signed the informed consent form. Study protocol was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences. Study was performed in compliance with the ethical guideline of the Helsinki Declaration for medical research involving human subjects.

Sample collection and 25-oxysterol level measurement

Two milliliters of blood was obtained from arterial sheath from patients being examined by angiography and two milliliters of venous blood was obtained from those who had been examined previously. More than 75% of patients were having coronary artery stenosis. Blood samples were transferred to the laboratory at endocrinology and metabolism research center at Taleghani Hospital. Plasma was separated by centrifugation and stored at -20°C for subsequent analyses.

Level of 25-oxysterol was measured by the enzyme-linked immunosorbent assay (ELISA) method (CUSABIOTECH, Wuhan, China). These patients were divided into two groups, one were receiving statins at the time of study and one not receiving

them. The relationship between receiving the drugs and taking these medications were investigated.

Statistical analysis

As the distribution of the data was normal (based on Kolmogorov- Smirnov test), the quantitative data were presented as mean ± SD and charts. Student's t-test was used to compare the means of two groups and one sample t-test was used for the comparison of the means of numerical data from population with the expected value of the normal population. For analysis of qualitative data, Chi-square and Fisher's exact tests were used. P-values less than 0.05 were considered as significant. All statistical analyses were performed using SPSS v.19.

Results

In the current study, 85 patients suffering from severe coronary artery stenosis were included. Fifty one males (60%) and 34 females (40%) were studied. Mean age of the subjects was 64.4 years (ranging from 40 to 87 years). About 58 (68%) patients were suffering from acute angina pectoris and 27 (32%) from chronic angina pectoris. Forty two (49.4%) patients were smokers and 3 (3.5%) had the history of stroke. Sixty six patients (87%) were taking atorvastatin.

In the current study patients' oxysterol level ranged from 19.37 to 29.8pmol/mol. Mean±SD of 25-hydroxy cholesterol level in 85 patients was 24.8±0.2.

No significant correlation between plasma oxysterols and smoking or history of stroke was observed ($p>0.05$), while taking atorvastatin was significantly correlated with plasma oxysterol levels ($p<0.001$; Table 1). Patients having history of receiving atorvastatin, but not taking medication in the study time, had significantly lower plasma oxysterol levels ($p<0.05$; Table 2).

Mean±SD level of the plasma oxysterol in patients was 24.8±0.2pmol/mL. This amount is significantly higher than that of the normal population which is estimated to be about 13.1pmol/mL. Interestingly, when they were assigned in quartiles, the mean level of first quartile (22.2pmol/ml) is significantly higher than the mean of the normal population (13.1pmol/ml). Mean level of oxysterol was significantly lower in patients receiving atorvastatin ($p<0.05$).

Discussion

Studying the molecular changes during atherosclerosis has always been important. In the current research we studied the plasma level of oxysterols, specifically 25-hydroxysterol as important derivatives of cholesterol metabolism, and its relationship with consumption of atorvastatin for the first time in Iranian population. Our re-

Table 1. Correlation between plasma oxysterol level with smoking, stroke and atorvastatin consumption

	n	%	Pearson correlation	p
Smoking	42	49.4	0.134	0.243
Stroke	3	3.5	0.188	0.930
Atorvastatin consumption	66	78	0.377	<0.001

Table 2. Mean plasma oxysterol level in patients according to atorvastatin consumption

History of atorvastatin consumption	Plasma oxysterol (Mean±SD) pmol/mL
Yes	24.4±2.10
No	26.38±1.60
Total	24.82±2.15

Table 3. Distribution of plasma oxysterol in patients; SD: standard deviation

	Frequency	Mean	Minimum	Maximum
First quartile	24	22.22	19.37	25.33
Second quartile	21	24.30	23.59	24.48
Third quartile	18	25.51	25.08	26.08
Fourth quartile	22	27.49	26.33	29.8
Total	85	24.79	19.37	29.8

search demonstrated that patients with more than 75% stenosis of coronary artery had higher level of plasma oxysterol. Cut-off for normal level of oxysterol in our population was 13pmol/ml (19, 20). In our study, all patients with 75% coronary artery stenosis had plasma oxysterol levels higher than 13pmol/mL. Mean level of hydroxysterol in 85 patients was 24.8±0.2pmol/mL. Low standard deviation proves high sensitivity of our measurements. In the study sample, minimum level of oxysterol was 19.37pmol/mL, and the maximum observed level was 29.84pmol/mL. These are significantly higher than normal amounts.

Kummerow et al. reported higher level of oxysterols in the plasma from coronary artery bypass grafting (CABG) patients compared to healthy age- and sex-matched controls. According to their study the concentration of sphingomyelin in the arterial cell membrane was directly correlated with the level of oxysterol in the plasma of patients suffering from severe atherosclerosis. This is expected to increase calcium influx required for producing the calcified type VII lesions in the coronary arteries (21). In 2008, a study by Endo et al. revealed that increasing the concentration of 7-ketosterols are associated with coronary risk factors in diabetic patients. They demonstrated that oxysterols are amongst the major causes of oxidation of low density lipoproteins (LDLs) and are implicated in the atherosclerosis process and, hence, development of cardiovascular diseases (22).

Findings of the current research are in compliance with Hodis (23), Zeiden (24) and Colles (25). Investigations by Hodis in 1991 and Zeiden in the same year in coronary artery patients revealed the role of oxysterols in degenerative diseases (23, 24).

Yasunobu et al. in 2001 demonstrated that coronary atherosclerosis is a reflection of oxysterols and the presence of auto-antibodies against LDL. Research by Yasunobu verified the relationship between oxysterols and coronary stenosis (20). Salonen et al. confirmed the relationship between oxysterols and the increase of carotid wall thickness (26). Results of the current study, replicate the results from these studies.

Another study by Qi Zhou et al. in 2000 showed that oxysterol level was higher in patients with more than 80% coronary stenosis (27).

Marcello Arca et al reported that the level of oxysterol in familial combined hyperlipidemic (FCHL) patients decreases with the use of anti-hyperlipidemic drugs (28). According to the data from current study using statins significantly reduce the level of plasma oxysterols. Level of oxysterol in patients receiving atorvastatin was significantly lower than patients not using it (24.4pmol/mL vs. 26.3pmol/mL; $p<0.001$).

Studies by Rikitake et al. and Sumi et al. demonstrated that Fluvastatin, another member of statin family, reduce the production of oxidizing radicals (29, 30). In the study by Giroux et al. it was demonstrated that simvastatin reduce the production of superoxide anions in a dose dependent manner (31).

In a study by Vasanahari et al. it was demonstrated that treatment with statins reduces the level of oxysterols in patients by 24% when compared to control untreated group (32). In the current study treatment by atorvastatin reduced the plasma oxysterols level from 26.4±1.6pmol/mL to 24.4±2.1pmol/mL ($p=0.001$).

In this study mean level of oxysterol was 24.8±2.17pmol/mL which is significantly more than the normal population's mean (23.1pmol/mL). Interestingly, when the samples were divided into quartiles, the mean plasma level of oxysterols in the first quartile (22.2pmol/mL) was significantly higher than population mean. Among the variables studied in the current study like CVA, smoking and receiving atorvastatin, oxysterol level was significantly reduced in those receiving atorvastatin. Contradictory results were achieved by Greet van Poppel et al. in 1997 demonstrating no relationship between plasma oxysterol levels with the

risk of developing atherosclerosis (33).

Although some risk factors did not show significant differences between two groups, clinical significance must be kept in mind in the interpretation of the results as an important factor. Patients with acute angina pectoris were having higher levels of oxysterols compared with patients with stable angina pectoris (25pmol/mL vs. 24.2pmol/mL, respectively) but the difference was not statistically significant (p=0.231).

Our study provided insight into the involvement of plasma oxysterols in the risk of developing coronary stenosis and the potential of atorvastatin for reducing plasma oxysterol level. Considering the experimental evidence regarding the adverse effects of oxysterols on endothelial cells in *in vitro* studies, results appear sound. However, due to some limitations due to study design, lack of controls and long term follow-up, our data could not be regarded as conclusive and further controlled trials are required for proving the findings.

Conclusion

In sum, the results showed that the level of plasma oxysterols is directly correlated with the advancement of coronary stenosis and its stage. Dramatic elevation of its level is predictive of severe stenosis. It appears that treatment with atorvastatin reduces the level of plasma oxysterols and the risk of stenosis. However, due to some limitations in the current study conducting controlled trials with larger sample sizes to prove this effect of atorvastatin is recommended.

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