Original Article

Trial of Atorvastatin on Serum Interleukin-6, Total Antioxidant Capacity, C-Reactive Protein, and Alpha-1 Antitrypsin in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Objective: The present study was designed to investigate the effects of atorvastatin on serum high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), total antioxidant capacity (TAC), and alpha-1 antitrypsin (AAT) in patients with chronic obstructive pulmonary disease (COPD). **Methods:** A clinical trial study conducted on 42 cases of COPD (Vali-Asr Hospital, Birjand, East of Iran, years 2014–16). Patients were randomly assigned to 21 controls and 21 cases who treated with atorvastatin (40 mg/day for 6 months). Inhaled corticosteroid and long-acting β-agonist were administrated in both groups. The trial was registered at the Iranian Registry of Clinical Trials (registration number: IRCT2016042527594N1). TAC was measured by ferric reducing/antioxidant power assay. An enzyme-linked immunosorbent assay was used to determine IL-6, AAT, and hs-CRP. Spearman's rho test and Wilcoxon, Mann–Whitney, paired, and independent t-tests were used for data analysis in SPSS 23. $P < 0.05$ was considered significant. **Findings:** A number of patients completed the study were 16 in atorvastatin and 18 in control group. Mean increments (μmol/L) of TAC (mean ± standard deviation [SD]) were 12.81 ± 605.25 ($P = 0.68$) in atorvastatin and 160.26 ± 280.54 ($P = 0.14$) in control group. Mean decrements of IL-6, CRP, and AAT (mean ± SD) were 1.41 ± 5.51 ($P = 0.71$), 0.98 ± 5.68 ($P = 0.72$), and 10.94 ± 46.83 ($P = 0.21$) in atorvastatin and 0.91 ± 11.70 ($P = 0.75$), 3.23 ± 7.00 ($P = 0.19$), and 18.77 ± 55.90 ($P = 0.21$) in control group. **Conclusion:** Atorvastatin did not succeed in maintaining TAC and CRP reduction. However, less reduction in AAT and more reduction in IL-6 in the atorvastatin group would be likely a beneficial effect in COPD.

Keywords: *Alpha-1 antitrypsin, Atorvastatin, chronic obstructive pulmonary disease, interleukin-6*

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Introduction

Chronic obstructive pulmonary disease (COPD) is a preventable and somewhat treatable condition characterized by airflow limitation that is not fully reversible. COPD is going to be the third cause of morbidity by 2020.[1] COPD and asthma are both with inflammatory basis. However, the effect of corticosteroids in the treatment of COPD is less prominent than in asthma.[2] Epidemiological studies have shown that inflammatory markers such as interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) affect prognosis of patients with COPD.[3] Oxidative process also involved in the pathogenesis and progression of the disease.[4] IL-6 is one of the factors considered in establishing pulmonary arterial hypertension (PAH) in patients with COPD.[5]

Statins are widely used in the treatment of hyperlipidemia and cardiovascular disease. However,

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multiple properties of statins make them suitable for condition aside from hyperlipidemia and cardiovascular disease.[6] Anti-inflammatory properties of statin have initially addressed by Albert *et al*., where statin use resulted in reduction of CRP levels in patients with cardiovascular disease.[7] Statins have also showed different effect on oxidative stress.[8] Atorvastatin can be effective in reducing IL-6. This may be an important anti-inflammatory property of statins in the treatment or prevention of PAH in patients with COPD.[9]

Statins may have potential therapeutic effects including PAH[10] in patients with COPD and therefore taken into consideration in recent years to modify the immune system and reduce inflammation in COPD.[11] Some studies have been proposed the benefits of statins such as reducing exacerbation, intubation, and mechanical ventilation,[12] and despite evidence of a role for statin drugs in patients with COPD, one systematic review suggests that findings must be confirmed by more trial study.[13]

The present study was designed to investigate the effects of statins on serum levels of CRP, IL-6, total antioxidant capacity (TAC), and alpha-1 antitrypsin (AAT) in patients with COPD.

Methods

The present study is a randomized clinical trial conducted on 42 known cases of COPD who were referred to Vali-Asr Hospital, Birjand, East of Iran, between 2014 and 2016. All cases had systolic pulmonary arterial pressure of >25 mmHg detected by echocardiograms and were performed in Vali-Asr Hospital by Vivid 10 echocardiography equipment (MEDISON, Korea). Since basically, the effect of atorvastatin on pulmonary artery pressure in COPD patients,[10] AAT, TAC of plasma, IL-6, and hs-CRP must be evaluated in our study, the sample size was calculated according to mean change comparison of systolic pulmonary artery pressure under the influence of pravastatin therapy: 47 ± 8–40 ± 6 in order of "α" = 0.05 and "β" = 0.1 extracted from a similar to our study (Lee *et al*.).[14] The required sample size was 21 in each group.

Patients were randomly assigned to one of two; the control and the case group [Figure 1]. At the end of 6 months, 16 patients in the atorvastatin group and 18 patients in the control group were remained to be eligible for completion of the study. The methods of our study were reviewed and approved by Birjand University of Medical Sciences (BUMS) Ethics Committee. All patients signed an informed consent form for the study. The trial was registered at the Iranian Registry of Clinical Trials with the registration number of IRCT2016042527594N1 where the full-trial protocol is accessible.

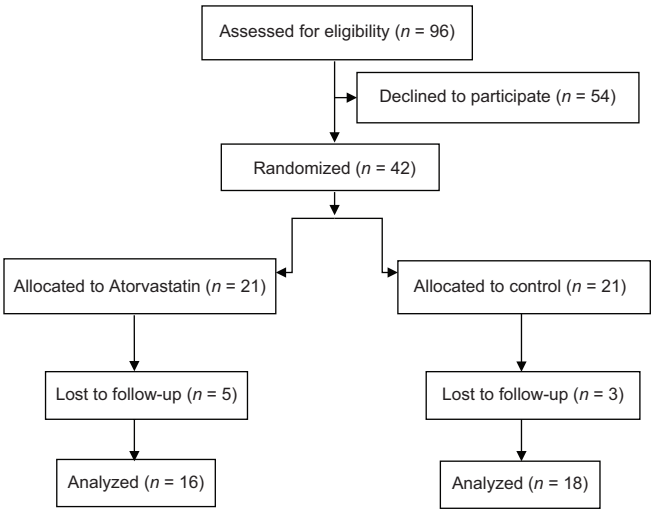

Figure 1: Study protocol flow chart

Inclusion criteria for the study were as follows: enrolling diagnostic criteria for COPD based on the American Thoracic Society (forced expiratory volume in 1 s [FEV1] <80% and FEV1/forced vital capacity <70%)[15] and regardless of disease duration and severity. Exclusion criteria were as follows: not treated with statins at least during the past 6 months, major comorbidities such as liver, renal, or heart disease, diabetes mellitus, and use of >2-week oral or parenteral corticosteroid (any dosage) over the past 2 years and any exacerbation which has led to hospitalization for >1 week during the study.

Before starting atorvastatin and also at the end of 24 weeks, venous blood samples (15 ml) were drawn from the case and control groups. Blood samples were collected in two separate tubes and sent to the laboratory. Serum in one of the tubes was used for measuring AAT and hs-CRP, and serum in another tube was centrifuged, separated into two microtubes, tagged, and frozen at −80°C to be used later to measure IL-6 and serum TAC.

After sampling, the patients in case group were treated with 40 mg/day of atorvastatin for the duration of 24 weeks. Routine treatment of COPD was similar for both groups and included inhaled corticosteroid (ICS), beclomethasone or fluticasone: 500–1000 μ/d, and long-acting β-agonist (LABA), salmeterol 25–50 μ/d, with or without the use of theophylline.

After completion of sample collection, all samples were defreezing simultaneously and prepared for IL-6 and serum TAC assay. Ferric reducing/antioxidant power assay was used to measure serum TAC (μmol/L). An enzyme-linked immunosorbent assay (Boster Biological Technology Co., California, USA) was used to determine IL-6 (pg/ml). AAT (mg/dl) and hs-CRP (mg/dl) were measured using INTEGRA 400 auto analyzer and

reagents from Roche Diagnostics, Germany. Inter- and intra-assay coefficient of variations for each kit is presented in Table 1.

The Statistical Package for the Social Sciences (SPSS) software version 23, (Parnian Engineering incorporated, Karaj, Iran) was used to perform statistical analysis. Descriptive statistics were used to express the percentage and means. The Shapiro–Wilk test is used for assessment of the normality of data. Paired sample and independent t-tests were used for comparison of the data with normal distribution and Wilcoxon and Mann–Whitney test for data with nonnormal distribution. Spearman's rho test was used to test the relationship between two continuous variables. $P < 0.05$ was considered statistically significant. Respectively, inter- and intra-assay coefficient of variations was 3.5% and 3.4% for AAT, 5.5% and 4.9% for IL-6, and 2.7% and 1.5% for hs-CRP kits.

Results

We had 16 patients in the atorvastatin and 18 patients in the control group at the end of 6 months who completed the study. The mean age among all patients was 64.72 ± 12.21 years (65.8 ± 11.5 years for atorvastatin group and 63.7 ± 7.6 years for control group; $P = 0.456$). Among all, 67.5% were female and 32.5% were male without significant difference in gender frequency between the two studied groups ($P = 0.54$).

Mean ± standard deviation (SD) level of hs-CRP, IL-6, serum TAC, and AAT at the beginning of study for both atorvastatin and control group has the same cohort comparable and presented in Table 1. Comparison of means at the end of the study did not likewise show any significant difference between two groups [Table 1].

The mean ± SD decrease of CRP from start to end of the study was 0.98 ± 5.68 ($P = 0.19$) in control group and 3.23 ± 7.00 ($P = 0.72$) in atorvastatin group. The mean ± SD increase of TAC from start to end of study was 160.26 ± 280.54 (median = +105.16) ($P = 0.14$) in control group and 12.81 ± 605.25 (median = +10.81) ($P = 0.68$) in atorvastatin group. Mean ± SD changes and median display in inflammatory markers within groups are presented in Table 2.

Significant positive correlations were observed between change in AAT and hs-CRP in control group at baseline ($r = 0.88$; $P < 0.001$) and at end of the study ($r = 0.49$; $P = 0.05$). Positive relationship was also observed between CRP and AAT in atorvastatin group before ($r = 0.82$; $P < 0.001$) and after ($r = 0.70$; $P < 0.001$) completion of the study. The relationship between serum levels of the other inflammatory markers is presented in Table 3.

Discussion

There was no significant difference in serum levels of inflammatory markers between atorvastatin and control group at the baseline. This means that with regard of inflammatory status, the two groups are identical at the baseline.

The reduction in IL-6 and hs-CRP was observed in both atorvastatin and control group. The possible explanation for reduction in hs-CRP and IL-6 serum levels in both groups may be partially related to additional attention in taking regular medications of ICS + LABA for COPD after entering the study. In this field, some studies have shown that steroids are efficient in reducing serum hs-CRP and IL-6 levels in patients with COPD.[16]

According to the present study, it can be seen that reduction of serum IL-6 level in the atorvastatin is more pronounced than in control group although the declining rate was not significant. IL-6 has a unique role in inflammatory diseases and targeting it could be considered for control of certain diseases.[17] In agreement with the present study, benefits of statin through IL-6 production inhibition are also shown in the study conducted by Young *et al*.[11] IL-6 is one of factors considered in establishing PAH in patients with COPD.[5] Anti-inflammatory properties of statins in the treatment or prevention of PAH in patients with COPD were also highlighted.[9,14,17] The beneficial effects of stains are also highlighted in a systematic review and meta-analysis of observational studies that emphasized a clear benefit of statins for patients suffering from COPD.[18] An *in vitro* study has shown that by inhibiting 3-hydroxy-3-methylglutaryl coenzyme

Table 1: Comparisons of inflammatory markers between atorvastatin and control group

Marker	Baseline (mean±SD)		*P*	End of study (mean±SD)		*P*
	Control	Atorvastatin		Control	Atorvastatin	
AAT	161.07±48.20	158.99±38.78	0.88*	142.30±27.68	148.05±37.39	0.59*
TAC	765.04±246.75	874.39±391.13	0.55**	925.30±329.24	887.20±377.87	0.74**
IL-6	5.28±8.30	3.22±5.32	0.25**	4.37±7.21	1.81±1.18	0.11**
hs-CRP	4.32±6.08	3.77±4.58	0.93**	1.08±2.28	2.79±4.66	0.09**

*Independent *t*-test, **Mann-Whitney test. AAT=Alpha-1 antitrypsin, TAC=Total antioxidant capacity, IL-6=Interleukin-6, hs-CRP=High-sensitivity C-reactive protein, SD=Standard deviation

Table 2: Mean levels and mean change of inflammatory markers within atorvastatin and control group

Marker	Mean±SD			Median	*P*
	Baseline	End of study	Mean changes		
Atorvastatin group					
AAT	158.99±38.78	148.05±37.39	−10.94±46.83	−7.35	0.21*
TAC	874.39±391.13	887.20±377.87	+12.81±605.25	+10.81	0.68**
IL-6	3.22±5.32	1.81±1.18	−1.41±5.51	+0.05	0.71**
hs-CRP	3.77±4.58	2.79±4.66	−0.98±5.68	+0.02	0.72**
Control group					
AAT	161.07±48.20	142.30±27.68	−18.77±55.90	−18.60	0.21*
TAC	765.04±246.75	925.30±329.24	+160.26±280.54	+105.16	0.14**
IL-6	5.28±8.30	4.37±7.21	−0.91±11.70	−0.05	0.75**
hs-CRP	4.32±6.08	1.08±2.28	−3.23±7.00	−0.45	0.19**

*Paired samples *t*-test, **Wilcoxon test. AAT=Alpha-1 antitrypsin, TAC=Total antioxidant capacity, IL-6=Interleukin-6, hs-CRP=High-sensitivity C-reactive protein, SD=Standard deviation

Table 3: The correlations between inflammatory markers in atorvastatin and control groups

Marker	AAT		TAC		IL-6	
	Baseline *r* (*P*)	End of study *r* t(*P*)	Baseline *r* (*P*)	End of study *r* (*P*)	Baseline *r* (*P*)	End of study *r* (*P*)
Atorvastatin group						0.21 (0.38)
TAC	0.23 (0.34)	0.30 (0.22)				
IL-6	−0.08 (0.73)	0.12 (0.63)	0.07 (0.76)	−015 (0.55)		
hs-CRP	0.82 (<0.001)	0.70 (<0.001)	0.18 (0.46)	0.32 (0.21)	0.06 (0.81)	
Control group						0.02 (0.94)
TAC	0.19 (0.46)	0.50 (0.85)				
IL-6	−0.28 (0.27)	0.06 (0.82)	0.17 (0.52)	−0.62 (0.01)		
hs-CRP	0.88 (<0.001)	0.49 (0.05)	0.24 (0.36)	0.16 (0.55)	−0.36 (0.17)	

r: Spearman's rho test. AAT=Alpha-1 antitrypsin, TAC=Total antioxidant capacity, IL-6=Interleukin-6, hs-CRP=High-sensitivity C-reactive protein

A reductase, atorvastatin lowered concentrations of several inflammatory molecules including IL-6 derived from basal-state endothelial cells.[19] Some researchers claimed that AAT is also able to suppress the synthesis of pro-inflammatory cytokines (including IL-6).[20,21] AAT showed less declining in the atorvastatin than in control group in our study so a possible suppressive effect on IL-6 and the issue may be considered as a beneficial effect of atorvastatin in patients with COPD. However, it should be also noted the less declining of hs-CRP and less increment of TAC in our atorvastatin group. Keeping in mind that hs-CRP is a marker of inflammation and TAC as body protector, beneficial effects of atorvastatin could not be confirmed in our study. To some extent, increased TAC in both groups is probably due to antioxidant effects of ICS in patient with COPD.[22] However, less increase in TAC in the atorvastatin group might be due to the inhibitory effect of atorvastatin on the production of antioxidant enzymes (coenzyme Q10). Coenzyme Q10 is probably one of the enzymes inhibited by atorvastatin, leading to the suppression of the antioxidant production.[23] In contrast to our finding, some researchers have shown the significant increase in antioxidant potential and decrease in CRP by taking atorvastatin.[24-26] To some extent, the inconsistency in results between our study and others may be related to the type of the selected patients and treatment duration with statin. In this regards, our study is essentially different from the study conducted by Buyukhatıpoglu *et al*. in patients with coronary artery disease[25] and from the study conducted by Soedamah-Muthu *et al*. in patients with type 2 diabetes for 1-year duration.[26]

An important point of our study was significant and positive relationship between hs-CRP and serum AAT both in atorvastatin and control groups. Both hs-CRP and AAT are known as positive acute phase proteins in systemic inflammation.[27] Interestingly, AAT is also labeled as an antiprotease and has anti-inflammatory effects.[28,29] A higher antitrypsin level in atorvastatin group is likely a protective factor in the body against the devastating effects of inflammation and oxidative stress.

One of our limitations was the small sample size and medium power of the study which was due dropped out cases from baseline. Because of small sample size, and lack of patients compliance the other limitations were:

The stage of COPD was not considered in this study and blood sampling was done only in two steps (beginning and end of the study). A strong point of the present study is concurrently assessment of several inflammatory markers in patients with COPD.

Although nonsignificant, our study suggests that concomitant use of atorvastatin with conventional therapy (ICS + LABA) in patients with COPD probably inhibits the production and release of IL-6 into circulation.

Considering the triad of inflammation, protease/antiprotease, and oxidation stress in the development of COPD, the possibility effects of atorvastatin would be considered in maintaining the AAT activity. However, atorvastatin failed in maintaining serum TAC and decreasing hs-CRP in patients with COPD.

Authors' Contribution

Anahita Arian, Sayyed Gholamreza Mortazavi Moghadam, and Toba Kazemi, contributed on idea, design, sample collection, patient management, and writing the manuscript. Mahmood Zardast and Asghar Zarban contributed on preparation laboratory tests and collaborated on writing the article. All authors read and approved the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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