

Full Length Research Paper

# Comparative Analysis of Aggressive vs. Conventional Atorvastatin Doses on IL-6 and NO Levels in Acute Myocardial Infarction Patients

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Accepted 14 September, 2024

High dose statin medication in acute coronary syndrome cases is a therapy which lowers mortality and morbidity rates. Interleukin-6 (IL-6) is produced in higher amounts in acute myocardial infarction (MI) and facilitates myocardial damage. However, secretion of nitric oxide (NO) is depleted. We aimed to compare the effects of conventional dose (10-40 mg/day) and aggressive dose (80 mg/day) atorvastatin medications on IL-6 and NO levels in patients with primary percutaneous transluminal coronary angioplasty (PTCA) intervention after acute MI. 50 patients (8 females, 42 males) with the diagnosis of acute MI with ST segment elevation enrolled to the study. Primary PTCA intervention was performed on these patients and consequently either conventional dose (10 to 40 mg/day) or aggressive dose (80 mg/day) atorvastatin medications were given to the patients. Three months later, plasma IL-6 and NO levels were determined and alterations in the groups were evaluated. IL-6 levels decreased from  $24.34 \pm 12.04$  to  $11.40 \pm 5.79$  pg/ml and from  $29.62 \pm 17.38$  to  $12.51 \pm 8.95$  pg/ml in conventional dose and aggressive dose regimens respectively ( $p < 0.001$ ). However, NO concentrations increased from  $22.90 \pm 8.24$  to  $31.70 \pm 7.56$   $\mu$ M in conventional dose and from  $19.37 \pm 5.60$   $\mu$ M to  $34.15 \pm 9.60$   $\mu$ M in aggressive dose groups ( $p < 0.001$ ). The effects of aggressive dose atorvastatin medication on IL-6 and NO levels were similar to conventional dose application in cases with ST segment elevation acute MI.

**Key words:** Atorvastatin, interleukin-6, nitric oxide, myocardial infarction.

## INTRODUCTION

Acute myocardial infarction (MI) is still an important cause of mortality and morbidity despite of advances in medical therapies and interventional cardiology in recent years (Sabel, 2000). The major pathophysiology of acute coronary syndromes (ACS) is summarized as vulnerable plaque, increased inflammation and hypercoagulability. For this reason, anti-inflammatory therapies such as statins and angiotensin converting enzyme inhibitors and anti-platelet therapies such as heparin and clopidogrel are primarily used in ACS without ST segment elevation and percutaneous intervention was postponed as possible

as until the cooling vulnerable plaque. On the contrary, physician should urgently decide to revascularization therapy which thrombolysis or percutaneous intervention in patient with ST elevation MI. Although, similar physiopathologic process was seen in both ACS and ST elevation MI, a major difference between two items is total coronary arterial occlusion and transmural infarction in ST elevation MI.

Statins are drugs which were proved to decrease mortality rates in ACS by great randomized controlled trials (RCT) (Braunwald et al., 2002). High dose statin medication becomes a routine application in most cardiology clinics independent of lipid profiles in ACS in recent years. However value of aggressive dose statin therapy in ST segment elevation acute MI remains

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unclear. Interleukins are inflammatory markers which provide communication between leukocytes.

IL-6 which is a pleiotropic cytokine is produced by dendritic cells, monocytes, macrophages, endothelial cells, osteoclasts, fibroblasts, mast cells and thymocytes. Leukocytes are infiltrated in damaged tissue in acute MI and monocytes are activated to secrete IL-1, IL-6, tumor necrosis factor (TNF- ) and interferon (Komsala et al., 2005). It was demonstrated that IL-6 was an immunoregulatory cytokine and proinflammatory key factor related with cardiovascular diseases (Chiappelli et al., 2005). Also, higher IL-6 levels are associated with increased MI risk (Ridker et al., 2000); whereas nitric oxide (NO) is a strong inhibitor of vascular smooth muscle contraction and adhesion of neutrophils to vascular endothelium. The protective role of NO for endothelium and the beneficial effects in ischemia reperfusion injury are well known. The bioavailability of NO decreases in occasions with increased oxidative stress like acute MI (Synder et al., 1992; Murad, 1999). Statins play a pivotal role in the recovery of endothelial dysfunction by supporting the IL-6 levels in acute MI investigated in a small number of researches (Treasure et al., 1995) . The reports derived from these studies did not tell us if the effects of aggressive dose statin application were superior to those of the conventional dose on NO and IL-6 levels, or if the beneficial effects of statins were clarified.

We aimed to compare the effects of conventional dose and aggressive dose atorvastatin therapy on IL-6 and NO levels in patients with ST segment elevation acute MI. Thus we targeted to show the applicability of low dose atorvastatin therapy in routine clinical use in acute MI.

## METHODS

### Study population

50 patients (42 males, 8 females) who were diagnosed as acute MI with ST segment elevation in Cardiology Department of Selcuk University Meram Medical Faculty were enrolled to this study. The protocol was approved by local ethical committee. Written informed consents of the patients were obtained. The patients who refused to cooperate were excluded from the study. The diagnosis of acute MI depended on clinical symptoms, characteristic electrocardiographic changes and elevated enzyme concentrations.

Patients were considered for enrollment in the study if they presented with ST elevation myocardial infarction, displayed > 1 mm ST segment elevation on two contiguous leads on the electrocardiography, and had a CK-MB elevated at least three times the upper reference limit on at least two determinations. In addition, troponin-I was measured on admission and with intervals of 8 h during 48 h and the patients with abnormal troponin-I levels -three fold elevation from the upper reference limit- were excluded from the study. We excluded the patients with renal failure on admission (serum creatinine level > 2.5 mg/dl); patients who reached the institution more than 12 h after onset of symptoms; patients who died before hospital discharge, and patients who were not eligible to primary percutaneous trans-luminal coronary angioplasty (PTCA) or patients with unsuccessful PTCA.

All study patients had single vessel disease. Because of needs to

use nitrates, the patients with multi vessels disease were excluded from the study. In addition, the patients with severe valvular heart disease, documented dilated cardiomyopathy, familial hypercholesterolemia, chronic pulmonary and hepatic disorders, patients with inflammatory disease, blood disorders, previous MI and coronary revascularization history were excluded from the study. Patients who were statin users before acute MI and nitrat users were excluded from the study.

### Study protocol

Venous blood samples were obtained from the patients in the first 24 h of application and 3 months after the diagnosis for the analysis of blood lipid, IL-6 and NO contents. The blood samples were aspirated into tubes with EDTA at 09 to 10 A.M and after centrifugation the plasma were separated and stored in eppendorf tubes at -80°C until analysis.

After the diagnosis of acute MI and PTCA intervention, atorvastatin medication (10 to 80 mg/day) was applied to the patients. The clinicians who were blinded to study were free for atorvastatin dose choice. Patients were included to the study groups randomly: One for aggressive dose and one for conventional dose. When each group reached to 25 participants, the study was terminated.

### Blood sample analysis

Total cholesterol analysis was performed with original Beckman reagents with the principle of coupled cholesterol esterase and cholesterol oxidase reactions on Synchron LX20 analysers ((Beckman Coulter, USA). LDL-cholesterol levels were calculated with Friedewald formula. IL-6 and NO analysis were performed with ELISA (Beckman Coulter,USA) and colorimetric methods (Cayman Chemical Company, USA), respectively.

### Statistical analysis

SPSS (version 15.0) was used for the statistical evaluation of the data. The results were given as  $\pm$  SD. Independent t test and chi-square tests were used for comparisons of parametric and categorical variables in the groups respectively. Paired t-test was performed for the comparisons of parameters before and after atorvastatin medication. Repeated measurement ANOVA was carried out for the evaluation of the inter group differences.  $p < 0.05$  is assumed as significant.

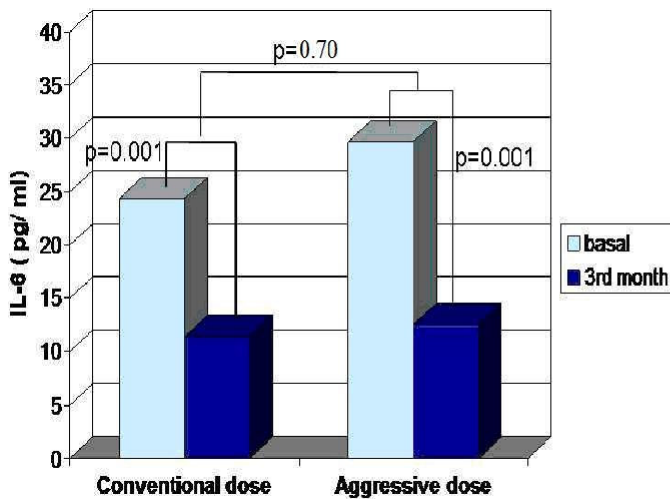
## RESULTS

The demographic characteristics of the patients were given in Table 1. While NO levels were  $22.90 \pm 8.24 \mu\text{M}$  before conventional dose atorvastatin regimen, IL-6 concentrations were  $24.34 \pm 12.04 \text{ pg/ml}$ . Before aggressive dose atorvastatin therapy, NO and IL-6 levels were  $19.37 \pm 5.60 \mu\text{M}$ ,  $29.62 \pm 17.38 \text{ pg/ml}$ , respectively. Baseline values of IL-6 and NO were similar in conventional dose and aggressive dose groups ( $p = 0.45$ , and  $p = 0.38$ , respectively).

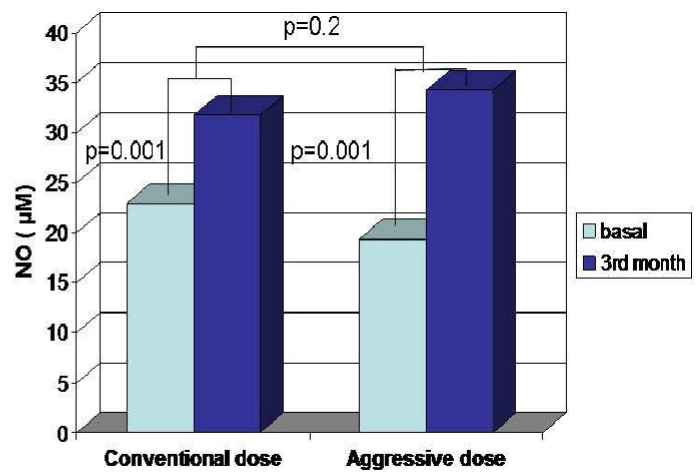
After statin therapy for three months, NO levels were increased in both groups. Also, IL- 6 concentrations were decreased in both groups (Figures 1 and 2). When these differences were compared between conventional dose

**Table 1.** Demographic characteristics of the study population.

Parameter	Conventional dose (n=25)	Aggressive dose (n=25)	P
Age	61.3±11.1	58.2±10.0	0.32
Sex (male/ female)	21/4	21/4	0.75
Mean body weight (kg)	61.3±11.1	59.2±9.7	0.48
Mean height (cm)	170.,0± 8.1	169,9±5.2	0.90
Body mass index (kg/m <sup>2</sup> )	27.3±4.1	26.5±3.6	0.52
Hypertension	17	15	0.70
Diabetes mellitus	9	11	0.76
Smoking	21	20	0.82
Drugs used	Beta blockers, Aspirin, Clopidogrel, ACE-inhibitors (except atorvastatin)	Beta blockers, Aspirin, Clopidogrel, ACE-inhibitors (except atorvastatin)	NS



**Figure 1.** IL-6 concentrations of the patients before and three months after atorvastatin application in conventional dose and aggressive dose groups. (IL-6 concentrations significantly decreased in both conventional and aggressive dose groups after atorvastatin application ( $p = 0.001$ ,  $p = 0.001$ ), however there was no significant difference between conventional dose and aggressive dose groups three months after atorvastatin application ( $p>0.05$ ).



**Figure 2.** NO concentrations of the patients before and three months after atorvastatin application in conventional dose and aggressive dose groups. (NO concentrations significantly decreased in both conventional and aggressive dose groups after atorvastatin application ( $p = 0.001$ ,  $p = 0.001$ , respectively), however there was no significant difference between conventional dose and aggressive dose groups three months after atorvastatin application ( $p>0.05$ ).

dose and aggressive dose groups, no significant difference was observed. Total cholesterol and LDL-cholesterol levels were decreased significantly (Table 2).

## DISCUSSION

We have demonstrated that aggressive dose atorvastatin medication lowered IL-6 levels and enhanced NO production similarly as conventional dose application in patients with acute MI with ST segment elevation for whom primary PTCA were carried out. The favorable effects of aggressive dose statin medication on mortality and morbidity rates in cases with acute coronary syndrome gave the result of routine aggressive dose

statin application in patients with acute MI with ST segment elevation; but there was no report which demonstrated the beneficial effects of aggressive dose statin application in the patient groups. Although clinical effects of this application were not evaluated in our study, we have shown that aggressive dose regimen was no superior to conventional dose on antiinflammatory and antioxidant status markers.

Ridker et al. (2000) evaluated plasma IL-6 concentration and occurrence risk for acute MI in 14916 men who seemed to be healthy. They pointed out that 202 participants who developed acute MI in follow up period showed higher IL-6 concentrations. Meredith et al. (2005) reported that IL-6 was more valuable and important in acute MI than unstable angina. Nawawi et al. (2004)

**Table 2.** The findings before and after atorvastatin therapy in conventional and aggressive dose groups and statistical evaluation.

Parameter	Conventional therapy		Aggressive therapy		P <sup>#</sup>
	Before atorvastatin	After Atorvastatin (3 months later)	Before atorvastatin	After atorvastatin (3 months later)	
IL-6 ( pg/ml)	24.34 ± 12.04	11.40 ± 5.79*	29.62 ± 17.38	12.51 ± 8.95*	0.70
NO (µM)	22.90 ± 8.24	31.70 ± 7.56*	19.37 ± 5.60	34.15 ± 9.60*	0.28
Total-cholesterol (mg/dl)	186.00 ± 42.85	131.67 ± 27.96*	193.92 ± 39.55	122.23 ± 27.30*	0.15
LDL-cholesterol (mg/dl)	121.53 ± 26.86	75.28 ± 24.45*	130.80 ± 31.29	70.60 ± 18.06*	0.39

\* Statistically significance level of intra-group change at the  $p < 0.001$  and it was derived by paired-t test. <sup>#</sup> Statistically significance of inter-group changes and it was obtained by repeated ANOVA test.

applied either 80 or 10 mg/day atorvastatin to total of 74 patients in order to investigate the effects of IL-6 levels on endothelial dysfunction in primary hypercholesterolemic patients. They have determined lipid and IL-6 concentrations 15 days, 3 and 9 months after the statin therapy. Significant decreases in total cholesterol, LDL-cholesterol and IL-6 levels and significant increases in HDL-cholesterol concentrations were observed in familial hypercholesterolemic patients with 80 mg/day atorvastatin application. Similarly, lipid parameters and IL-6 levels decreased in nonfamilial hypercholesterolemic patients with 10 mg/day atorvastatin therapy. This study pointed out that either low or high dose atorvastatin application in patients with primary hypercholesterolemia suppressed endothelial activation by early recovery of endothelial dysfunction and inhibition of inflammatory response, independent of their lipid lowering effects. These findings suggested that inflammatory response and endothelial dysfunction might be minimized by statin therapy in patients both with atherosclerotic vascular disease and with hypercholesterolemia and high risk for development of atherosclerotic events.

Marketou et al. (2006) investigated the effects of atorvastatin and simvastatin medications on SICAM, TNF- and in hyperlipidemic patients and demonstrated that their anti-inflammatory and antioxidant activities initiated 2 h after statin ingestion. In another study performed on 24 patients with acute MI with ST segment elevation, the researchers observed 10 mg/day atorvastatin therapy caused a 40% decrease in IL-6 levels compared to placebo during six weeks follow up (Stefanadi et al., 2009). We determined that with atorvastatin application for 3 months duration, IL-6 levels significantly decreased in both groups ( $p = 0.001$ ).

However aggressive dose did not make an additional benefit on IL-6 levels. Di Napoli et al. (2005) showed that statins decreased myocardial ischemia. It was demonstrated by some other researchers that statins played a pivotal role in activation of nitric oxide synthase (NOS) (Laufs et al., 1998; Shiga et al., 2005) and these findings were supported by two different investigation which concluded that endothelial NOS (eNOS) production was

regulated by statins and they also suppressed inflammatory response (Naito et al., 2006; Naidu et al., 2003). Anti-inflammatory effects of 10 mg/day atorvastatin were observed in Nagasaki et al. (2006) in high cardiovascular risk patients. In another research, the effects of statins on endothelial function and the bioavailability of NO in 41 hypercholesterolemic patients (with LDL-cholesterol levels of  $>130$  mg/dl) independent from their lipid lowering effects were investigated (John et al., 2005). They observed significant increases in NO levels resulting from the stimulation of NOS after 20 mg/day atorvastatin application. In our study, 10 to 80 mg/day atorvastatin application for 3 months caused significant increases in NO production ( $p < 0.001$ ). This significant increase in NO production which is assumed as an endogenous anti-inflammatory agent probably resulted from the stimulation of eNOS. Further investigations through this pathway may be beneficial. The clinical use of atorvastatin maintains anti-inflammatory effects via either IL-6 or NO and this fact makes atorvastatin application preferable however aggressive dose does not seem to give any additional benefit.

Baldassarre et al. (2005) applied 10 mg/day and 20 to 40 mg/day atorvastatin therapy for 3 months on 30 and 24 patients with coronary artery disease respectively. They observed 22.8, 32.6 and 19% decreases in total cholesterol, triglyceride and LDL-cholesterol levels respectively in 10 mg/day atorvastatin group. Similar effects were demonstrated in 20 to 40 mg/day group and the % decreases in the same parameters were as follows: 28.3, 35.3 and 33.6%. They concluded that low dose atorvastatin application was also effective. During the course of AMI, total cholesterol and LDL-cholesterol tended to decrease (25 to 30%) in the first 48 to 72 h and in some patients these low levels persist for 6 to 12 months. The mechanism of this early response is not clear but the antilipidemic effects of suggested to be evaluated by determination of lipid concentrations in 1 to 3 months of application. Anyway atorvastatin medication for 3 months provided 33.33 and 42.34% decrease in total cholesterol and LDL-cholesterol levels respectively, and these findings are in concordance with the previous

results.

## Limitations

The small number of the patients is one of the most important limitations. The duration of medication can be discussed but there is a report which declared that IL-6 levels decreased at the end of atorvastatin therapy for six weeks duration (Stefanadi et al., 2009). Another limitation is that because of our exclusion criteria like unsuccessful PTCA, thrombolytic therapy, previous MI and revascularization history, our results can not be generalized to all patients with MI with ST elevation. Moreover because of patients who have been statin users at the time of admission were excluded from the study, optimal statin dose in this population still remains unclear.

## Conclusion

We conclude that the obvious alterations in NO and IL-6 levels by means of atorvastatin application designate their anti-inflammatory contribution in treatment protocol. However we suggest a modest dose choice insofar as atorvastatin application according to the clinical and laboratory findings of the patients.

## ACKNOWLEDGEMENT

The research was funded by Scientific Investigations and Projects Coordination of Selcuk University grant (Project Number: BAP06102008).

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