

EFFECT OF ATORVASTATIN IN LIPID PROFILE CHANGES AND INFLAMMATION MARKER ON DIABETES PATIENT WITH DYSLIPIDEMIA

Wulan Panduwi Melasari^{1*}, Suharjono², Wiwid Samsulhadi³

¹Clinical Pharmacy Master Program, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

²Department of Clinical Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

³Haji Hospital, Surabaya, Indonesia

ABSTRACT

Diabetes is one of the risk factors for cardiovascular disease (CVD). Diabetics patients have 2 to 4 times increased risk of cardiovascular disease compared with non-diabetics. TNF-alpha is a proinflammatory cytokine that can be used to determine the risk of atherosclerosis complications triggered by inflammation in diabetes. Statins are a class of HMG CoA reductase inhibitors that inhibit cholesterol biosynthesis and have pleiotropic effects that inhibit the release of inflammatory cytokines like TNF-alpha and stabilize atherosclerotic plaques. This study aims to determine the effect of atorvastatin 20 mg/day for 30 days in reducing the lipid profile and TNF-alpha inflammatory markers in patients with diabetes dyslipidemia. Diabetes patient with dyslipidemia who included the inclusion criteria in this observational prospective cohorts studies treated with atorvastatin for 30 days (n = 19). The efficacy of statin therapy was measured by lipid profiles (LDL, TG, HDL, and total cholesterol) and TNF-alpha. The results of the study showed that atorvastatin decreased 40.55% of LDL levels, 15.34% of TG levels, and 30.70% of total cholesterol levels which statistically significant (P <0.05). As for HDL, there is an increase of 6.06% but statistically non-significant (P >0.05). TNF-alpha levels increased by 11.30% which statistically non-significant (P >0.05). The use of atorvastatin 20 mg for 30 days gave reduction in LDL, TG, and total cholesterol and increased in HDL. Atorvastatin does not have a reducing effect on TNF-alpha. There was no correlation between lipid profile changes with TNF-alpha changes.

Keywords: atorvastatin; lipid; TNF-alpha; diabetes; dyslipidemia

ABSTRAK

Pasien diabetes memiliki 2 sampai 4 kali peningkatan risiko penyakit kardiovaskular dibandingkan dengan non diabetes. Untuk mengetahui risiko terhadap adanya penyakit kardiovaskular dapat dilakukan pemeriksaan petanda inflamasi TNF-alpha yang merupakan sitokin proinflamasi yang dapat memicu terjadinya aterosklerosis. Statin merupakan golongan HMG CoA reductase inhibitor yang menghambat biosintesis kolesterol dan memiliki efek pleiotropik sehingga menghambat pengeluaran sitokin inflamasi seperti TNF-alpha dan menstabilisasi plak aterosklerosis. Pada penelitian ini bertujuan untuk mengetahui pengaruh atorvastatin 20 mg/hari selama 30 hari dalam menurunkan profil lipid dan petanda inflamasi TNF-alpha pada pasien diabetes dislipidemia. Pasien DM tipe 2 dengan dislipidemia yang memenuhi kriteria inklusi dilakukan studi observasional prospektif kohort dan mendapat atorvastatin selama 30 hari (n=19). Efektivitas terapi statin diukur melalui pemeriksaan profil lipid (LDL, TG, HDL, dan kolesterol total) dan petanda inflamasi TNF-alpha. Hasil penelitian didapatkan pada terapi atorvastatin terjadi penurunan untuk LDL sebesar 40,55%, TG 15,34%, dan kolesterol total 30,70% yang signifikan secara statistik (P <0,05). Sedangkan untuk HDL, terjadi peningkatan sebesar 6,06% yang tidak signifikan secara statistik (P >0,05). Untuk nilai TNF-alpha terjadi peningkatan sebesar 11,30% yang tidak signifikan secara statistik (P >0,05). Dari penelitian ini disimpulkan bahwa pemakaian atorvastatin 20 mg selama 30 hari memberikan penurunan terhadap LDL, TG, dan kolesterol total dan terhadap HDL terjadi kenaikan. Atorvastatin tidak memberikan efek penurunan terhadap TNF-alpha. Tidak ada korelasi antara perubahan profil lipid dengan perubahan TNF-alpha.

Kata kunci: atorvastatin; lipid; TNF-alpha; diabetes; dislipidemia

Correspondence: Wulan Panduwi Melasari, Clinical Pharmacy Master Program, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. E-mail: wulanpanduwi21@gmail.com

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases with characteristics by hyperglycemia that occurs due to abnormalities of insulin secretion, insulin action or both (PERKENI 2015). Based on the results of basic health research in 2013, diabetes is one of the risk factors for cardiovascular disease (CVD) (Ministry of Health 2013). Diabetic patients have 2 to 4 times increased risk of stroke and cardiovascular disease compared with nondiabetes (Powers 2015). Dyslipidemia in diabetic patients occurs because of insulin resistance that causes lipid disorders. Lipid profiles in patients with type 2 diabetes are usually characterized by hypertriglyceridemia, decreased HDL, and elevated LDL (Wu & Parhofer 2014).

American Diabetes Association (ADA) recommend the target of LDL level in high-risk diabetic patients with cardiovascular risk factors less than 70 mg/dl. If this target can not be achieved, another alternative is chosen by lowering LDL 30% -50% of initial value. In high-risk patients with cardiovascular risk factors, high-intensity statin was used as therapy for more significant LDL reduction (ADA 2017).

One of the therapy used to lower LDL is atorvastatin. Atorvastatin classified as moderate intensity at 10-20 mg doses and high intensity at 40-80 mg doses (ADA 2017). In addition, statins have anti-inflammatory, antithrombotic properties, and the ability to stabilize atherosclerotic plaques (Davignon 2004). Statins can decrease the expression and function of molecules on the surface of leukocytes by inhibiting migration and chemotaxis from neutrophils to the endothelium thereby inhibiting the release of proinflammatory cytokines TNF-alpha (Stancu & Sima 2001). TNF-alpha is a proatherogenic cytokine produced by several cells involved in the process of atherosclerosis including macrophages, endothelial cells and smooth muscle cells (Tousoulis et al 2016).

Atorvastatin has a half-life of 14 hours and has an active metabolite with potent equivalent to its parent compound to inhibit HMG-CoA reductase. The longer actions of atorvastatin providing a greater reduction in cholesterol synthesis (Schachter 2005). This study was conducted to determine the efficacy of atorvastatin 20 mg to decrease the basic lipid profile in the form of total cholesterol, triglycerides, HDL and LDL and TNF-alpha inflammatory markers in DM patients with dyslipidemia. This study was conducted for 30 days according to the maximum effect of cholesterol and triglyceride reduction in 2-4 weeks of therapy

(American Society of Health System Pharmacist 2011, Lacy et al 2016).

MATERIALS AND METHODS

This study is a prospective observational study of cohorts. Patients with type 2 diabetes with dyslipidemia in the Internal Medicine Polyclinic of Haji Hospital, Surabaya during November 2017 - January 2018 who included into the inclusion criteria were patients aged > 21-75 years, lipid profiles of LDL > 100 mg/dL, TG > 150 mg/dL, did not receive statin therapy 7 days earlier and had signed informed consent. Exclusion criteria from this study were patients with increased liver function tests, patients with severe chronic kidney disease to renal failure, patients receiving anti-inflammatory medication, patients with infectious disease, inflammation, malignancy, and autoimmune disease. Examination of LDL, TG, total cholesterol, HDL were performed at General Hajj Hospital Laboratory. TNF-alpha examination using ELISA (Biolegend®) methodology was performed at the Infection Specialized Hospital Surabaya. Paired t-test and Wilcoxon test were used to determine differences in lipid, IL6 and TNF-alpha profiles before and after treatment using. The correlation between lipid profile and inflammatory markers TNF-alpha before and after therapy analyse with Pearson correlation test. The result of statistical test is significant if $p < 0,05$ with confidence interval equal to 95%.

RESULTS

A total of 19 patients who met the inclusion criteria were used as the subjects of the study. Patient demographic data can be seen in table 1. Research subjects consisted of 13 women (68.42%) and 6 men (31.58%), with mainly in 46-59 years group (57.89%) and based on BMI 57.89% in 18,5-24,9 kg/m² group. The comorbid disease are hypertension 10 patients (52.63%) and uric acid 7 patients (31.58%).

Table 1. Early characteristics of diabetes dyslipidemic patients

Characteristics of Patients	N	Percentage (%)	Average ± SD
Gender	Men	6	31.58
	Woman	13	68.42
Age Range (years old)	26 - 45	1	5.26
	46 - 59	11	57.89
	60 - 74	7	36.84
BMI (kg/m ²)	18.5 - 24.9	11	57.89
	25 - 29.9	7	36.84
	>30	2	10.53
Comorbid	Hypertension	10	52.63
	Uric acid	7	31.58

There was a reduction of LDL level with mean value of pre 163.47 ± 28.77 mg/dL and post 97.89 ± 29.71 mg/dL, reduction of TG with mean value of pre 161.89 ± 76.12 mg/dL and post 132.21 ± 75.48 mg/dL, and cholesterol total with mean value of pre 232.84 ± 43.19 mg/dL and post 161.89 ± 44.73 mg/dL. There was

significantly decreased in LDL, TG, and total cholesterol ($P < 0.05$), whereas an increase in HDL with a mean value of pre 49.00 ± 10.75 mg/dL and post 51.11 ± 9.28 mg/dL did not increase significantly ($P > 0.05$). Results of TNF-alpha with mean values of pre 9.89 ± 3.86 pg/mL and post 161.89 ± 44.73 pg/dL.

Table 2. Effect of atorvastatin on lipid profile and inflammatory marker TNF-alpha on diabetes patients with dyslipidemia for 30 days

Parameter	Pre	Post	Average Difference	% Changes	Significance
LDL (mg/dL)	163.47 ± 28.77	97.89 ± 29.71	65.58	40.12	0.00 ^a
TG (mg/dL)	161.89 ± 76.12	132.21 ± 75.48	29.68	15.34	0.026 ^a
HDL (mg/dL)	49 ± 10.75	51.11 ± 9.28	2.11	6.06	0.25 ^b
Total Cholesterol (mg/dL)	232.84 ± 43.19	161.89 ± 44.73	70.95	30.47	0.00 ^a
TNF-alpha (pg/mL)	9.89 ± 3.86	11.01 ± 4.42	0.79	7.09	0.49 ^a

Table 3. Lipid profile based on NCEP ATP III

Parameter	Category	Pre Therapy		Post Therapy	
		Total	%	Total	%
Total Cholesterol	Desirable (<200 mg/dL)	3	15.79	18	94.74
	Borderline high (200-239 mg/dL)	10	52.63	-	-
	High (≥ 240 mg/dL)	6	31.58	1	5.26
LDL	Optimal (<100 mg/dL)	-	-	11	57.89
	Near optimal (100-129 mg/dL)	-	-	7	36.84
	Borderline high (130-159 mg/dL)	10	52.63	-	-
	High (160-189 mg/dL)	7	36.84	-	-
	Very high (>190 mg/dL)	2	10.53	-	-
TG	Normal (<150 mg/dL)	7	36.84	14	73.68
	Borderline high (150-199 mg/dL)	9	47.37	3	15.79
	High (200-499 mg/dL)	3	15.79	2	10.53
	Very high (≥ 500 mg/dL)	-	-	-	-
HDL	<40 mg/dL	4	21.05	1	5.26
	41-59 mg/dL	11	57.89	14	73.68
	≥ 60 mg/dL	4	21.05	4	21.05
Total (n)		19 patients			

Table 4. Results of correlation test of TNF-alpha changes to lipid profile changes in diabetes dyslipidemic patients

Parameter	Correlation coefficient (r)	Significance (p)
TNF-alpha	LDL	0.139
	TG	0.597
	HDL	0.336
	Total Cholesterol	0.896

The results of the correlation analysis showed no correlation between the lipid profile and TNF-alpha ($P > 0.05$).

DISCUSSION

uring the three months of the study period, 20 patients included the inclusion criteria with 1 patient dropped out. Demographic data show the age of diabetics is the most common in 46-59 years as 57.89% (Table 1). From epidemiological study, patients with diabetes are most commonly by the productive age of between 40-60 years in developing countries (Shaw et al 2010). It was found that hypertension was the most common comorbidity in 52.63% research subject, followed by 31.58% of uric acid (Table 2). Hypertension is twice more common in diabetic patients as compared to non-diabetics. Hypertension in diabetics has a persistent BP value of $\geq 140/90$ mmHg. In the EUROASPIRE IV survey only 54% of diabetic patients achieved BP levels of less than 140/90 mmHg (Grossman & Grossman 2017).

Insulin resistance accompanied by increase free fatty acid into the liver play an important role in increasing the incidence of diabetes dyslipidemia, characterized by high TG concentrations, low HDL concentrations, and high small dense LDL concentrations (Chehade et al 2013). American diabetic association (ADA) and PERKENI recommended that the target lipid profile for patients with diabetes is LDL <100 mg/dl (2.60 mmol/l), HDL <40 mg/dl (1.02 mmol/L, and TG <150 mg/dl (1.7 mmol/l) (ADA 2017, PERKENI 2015).

The NCEP-ATP III Guideline has classified lipid profile based on each levels. Based on guideline, LDL levels are divided into five groups. There is optimal (<100 mg/dL); near optimal (100 - 129 mg/dL); borderline high (130 - 159 mg/dL); high (160 - 189 mg/dL); and very high (≥ 190 mg/dL). TG levels divided into four groups. There is normal (<150 mg/dL); borderline high (150 - 199 mg/dL); high (200 - 499 mg/dL) and very high (≥ 500 mg/dL) (NCEP-ATP III 2002).

Patients who met the inclusion criteria were measured on the lipid profile before treatment as baseline values. The results of lipid profile from 19 patients showed that for total cholesterol (Table 2) the mean value of pre 232.84 ± 43.19 mg/dL and post 161.89 ± 44.73 mg/dL decreased by 30.70%. For baseline total cholesterol levels (Table 2) were distributed mainly in borderline high (200-239 mg/dl) as 52.63%, high (? 240 mg/dL) as 31.58%.

In the LDL results (Table 2), the mean value of pre 163.47 ± 28.77 mg/dL and post 97.89 ± 29.71 mg/dL decreased by 40.55%. For baseline values of patients (Table 3) mostly in the high borderline range (130-159

mg/dl) as 52.63%, high (160-189 mg/dl) as 36.84%. In diabetic patients there is an increase small dense LDL which more atherogenic that causes risk factor for cardiovascular disease (NCEP-ATP III 2002). For the TG value (Table 2), the mean value of pre 161.89 ± 76.12 mg/dL and post 132.21 ± 75.48 mg/dL decreased by 15.34%. Baseline values of patients (Table 3) mostly located in borderline high (150-199 mg/dL) as 47.37%. For the HDL value (Table 2), the mean value of pre 49.00 ± 10.75 mg/dL and post 51.11 ± 9.25 mg/dL increased by 6.06%. Baseline values of patients (Table 3) <40 mg/dl as 21.05% and ≥ 60 mg/dL as 21.05%.

After 30 days of atorvastatin therapy, there was a change of total cholesterol; desirable range (<200 mg/dL) as 94.74%. In the results of LDL, the most patients were found in the optimal range (<100 mg/dL) as 57.89% and near optimal (100-129 mg/dL) as 36.84%. For the TG value, the most patients were in the normal range (<150 mg/dL) as 68.42%, but there were still patients in the borderline high range (150-199 mg/dL) as 10.53% and high (200-499 mg/dL) as 10.53%. For the HDL values, there are still patients with range <40 mg/dL as 5.26%. Based on the change of lipid profile, statistical analysis used a paired t-test for the data before and after therapy with a significant decrease in LDL, TG, and total cholesterol ($P < 0.05$), but no significant increase in HDL value ($P > 0.05$).

Based on previous study, ATGOAL showed a decrease in LDL-C targeted at week 4 for low, medium, high-risk patients ($n = 1049/1246$) with percentage of patients achieving LDL-C target of 91.9% , 87.6%, and 79.2% for each category with a total of 84.2% of patients achieving LDL-C target and did not require dose titration (McKenney et al 2005). The Thai ATGOAL study also showed that 81.6% of patients and 87.1% of atorvastatin therapy ($n = 240$) had achieved LDL targets at week 2 and 4 (Deerochanawong et al 2007).

Atorvastatin also have pleiotropic effects by inhibiting isoprenoid intermediates such as FPP and GGPP causing obstacles to isoprenyl GTPases such as Ras, Rho, and Rac leading to an inactivated Ras and Rho accumulation in the cytoplasm, where Rac causes an increase in NADPH oxidation causing an increase in ROS (Zhou & Liao 2010). ROS increases the translocation of NF- κ B p65 subunits by transcribing the pro-inflammatory genes MCP-1, Selectin, VCAM-1, and ICAM-1. This event facilitates monocyte adhesion to endothelial vascular, rolling, and diapedeses in sub-endothel. TNF- α secretions of active macrophages will maintain an increase in adhesion molecules by increasing NF- κ B signaling in the endothelium and

promoting smooth muscle cell growth and proliferation leading to atherosclerosis. Therefore, atorvastatin 20 mg for 30 days is expected to provide pleiotropic effect by inhibiting the release of inflammatory cytokines TNF-alpha to prevent atherosclerosis (Paneni et al 2013).

In this study, the mean values of pre TNF-alpha 9.89 ± 3.86 pg/dL and post 11.01 ± 4.42 pg/dL increased by 11.30% which statistically non-significant ($P > 0.05$). In the previous study, TNF-alpha values obtained in normal patients were 4.46 ± 1.42 pg/dL (Goyal et al 2012). Another study showed TNF-alpha levels were $9.28 (7.56-11.40)$ pg/dL ($n = 15$) and $10.02 (5.16-14.69)$ pg/dL ($n = 14$) in diabetic patients with dyslipidemia (Bellia et al 2010).

Patients who did not achieved fasting glucose and post prandial glucose levels as intended may cause hyperglycaemia. This condition may increase the proportion of FFA that activates TLR resulting in translocation of NF-kB which produced inflammatory cytokines such as TNF-alpha. Increased TNF-alpha may cause a decrease in IRS-1 leading to insulin resistance (Paneni et al 2013).

In obese patients, increased insulin resistance commonly associated with the increased amount of visceral adipose tissue. Abdominal fat tissue has a large lipolysis rate and insulin resistance, resulting in increased free fatty acid production. High free fatty acids will transport to the liver and converted to VLDL and will increase insulin insensitivity. Abdominal fat tissue also triggers an increase in the production of several inflammatory mediators such as TNF-alpha. Inflammatory mediators also contribute to increased peripheral tissue insulin resistance (Triplitt and Reasner 2011).

CONCLUSION

The results of this study showed that therapy of atorvastatin 20 mg for 30 days reduced LDL, TG, total cholesterol values and increased HDL and TNF-alpha values. There was no correlation between changes in lipid profile values (LDL, TG, total cholesterol, HDL) with TNF-alpha value.

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