Research Article

Effect of statins on lipoprotein (a) in dyslipidemic patients

Joel Bijou Irudayam*, R. Sivaraj, P. Nirmala

Department of Pharmacology, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India

Received: 15 September 2014
Accepted: 23 September 2014

*Correspondence to:
Dr. Joel Bijou Irudayam,
Email: joeswatmail@gmail.com

ABSTRACT

Background: Elevated plasma lipoprotein (a) (Lp(a)) levels, which act synergistically with low-density lipoprotein cholesterol (LDL-C), are an independent risk factor for cardiovascular diseases (CVD). The effect of statin drugs on Lp(a) levels has not been well-demonstrated in clinical studies. This prospective, randomized, comparative clinical study with parallel treatment groups was conducted to assess the effect of simvastatin, atorvastatin and rosuvastatin, on serum Lp(a) levels and serum lipid profile, in treatment-naive dyslipidemic patients without CVD.
Methods: A 12 weeks study, with 85 patients, aged 40-70 years, diagnosed with borderline high LDL-C, were assigned to three groups with their informed consents. Group A (n=28) was treated on simvastatin 20 mg/day; Group B (n=29) on atorvastatin 10 mg/day; and Group C (n=28) on rosuvastatin 5 mg/day. Patients' lipid profile and Lp(a) levels were assessed at 0, 4th and 12th week of treatment period. Statistical analysis was done using Duncan's test (p<0.05) and one-way ANOVA (p<0.001).
Results: At the end of 12 weeks, serum Lp(a) reduction was substantial at 18.73% in atorvastatin group; at insignificant levels (3.15%) in simvastatin group, whereas an elevated level of 8.58% in Lp(a) was recorded in rosuvastatin group. All three treatment groups showed a significant positive impact on the lipid profile. No adverse drug reactions were reported.
Conclusion: The impact of statin monotherapy on lipid profile doesn't correlate with its effect on Lp(a). Atorvastatin has shown a significant reduction in Lp(a) unlike the other statins, and should be preferred in patients with increased risk of CVD.

Keywords: Lipoprotein (a), Dyslipidemia, Simvastatin, Atorvastatin, Rosuvastatin

INTRODUCTION

Cardio vascular disease (CVD), is the leading cause of mortality in India and the second most common cause worldwide.[1,2] Due to its increased incidence, the National and International guidelines on cardiac health, target a down-regulation of low-density lipoprotein cholesterol (LDL-C) as a treatment for atherosclerotic CVD.[3,4] But after the CARE study noted that many patients suffering from cardiovascular events have normal plasma cholesterol levels,[5] the need to identify non-classical and independent risk factors of CVD and develop suitable therapies to minimize their risk became a part of cardiovascular research.

Lipoprotein (a) (Lp(a)) is a circulating lipoprotein composed of liver-derived apo(a) covalently bound to apo(b). It is also similar in lipid composition to apo(b) of LDL.[6] The PRIME study established Lp(a) as a predictor of coronary heart disease.[7] Later increased Lp(a) (>30 mg/dl in many studies) was identified as an independent risk factor for CVD.[8-10]

Elevated Lp(a) levels in plasma act synergistically with those of LDL-C to increase CVD risk.[11,12] The LDL-C lowering effect of HMG CoA reductase inhibitors (statins) has been well-documented, but the effect of statins on Lp(a) levels has not been well-established by clinical studies. Although there are some studies that show combination therapies of statins and niacin to reduce Lp(a) levels,[13,14] Statin monotherapy is here to stay as the preferred treatment of hyperlipidemias and prophylaxis for CVD in developing countries like India. Contradictory findings have been reported concerning variations in Lp(a) levels after statin treatment.[15-18] This calls for a better understanding of statin monotherapies on Lp(a) levels.

Objectives

The following study is aimed at a comparative analysis of the effect of three major statins; simvastatin, atorvastatin, and rosuvastatin, on serum lipid profile and Lp(a) levels among dyslipidemic patients without CVD.

METHODS

Study population

A prospective, randomized, open-label interventional study was conducted in treatment-naive dyslipidemic patients visiting the tertiary care center (Rajah Muthiah Medical College and Hospital, Chidambaram, Tamil Nadu, India) for a routine master health check-up. Patients were screened to assess their eligibility to participate in the study.

Inclusion criteria

1. Patients aged between 40 and 70 years
2. Patients who have read, signed, and agreed to the items listed in the informed consent form
3. Patients diagnosed with dyslipidemia with borderline high LDL-C (National Cholesterol Education Program, Adult Treatment Panel-III guidelines).[3]

Exclusion criteria

1. Patients having an allergy, hypersensitivity, or intolerance to statins or their derivatives
2. Any patient who has a previous history of substance abuse or dependency
3. Pregnant/lactating women
4. Patients with a history of any of the following:
   - Hepatic or renal impairment
   - Presence of uncontrolled or untreated hypertension or diabetes mellitus
   - Cardiovascular diseases like congestive heart failure. The presence of CVD was assessed by the World Health Organization protocol, which includes a detailed questionnaire and 12-lead electrocardiography
   - Active cancer within the last 5 years or a diagnosis of cancer within the last 5 years
   - Fibromyalgia, myopathy, rhabdomyolysis, unexplained muscle pain or weakness, and/or discontinuation of a statin because of myalgia.

Materials and Methods

The study was approved by the Institutional Human Ethics Committee and was carried out strictly in accordance with International Conference on Harmonization Good Clinical Practice guidelines. Informed consent was obtained from all the patients before their enrollment into the study. It was a 12 weeks study with 85 patients who fulfilled the eligibility criteria. They were randomly assigned to one of the three treatment groups. Group A (n=28) received simvastatin 20 mg once daily (OD); Group B (n=29) received atorvastatin 10 mg OD; and Group C (n=28) received rosuvastatin 5 mg OD. The dosage for each statin in this study was determined based on previous dose efficacy studies.[19-21] All the study drugs were generic products marketed by Micro Labs

Limited, Bangalore, India. The patients were asked to take their study medication every day at bedtime after food. The following flowchart illustrates the movement of study patients through the study (Figure 1).

All patients in the study received their respective study drug for a period of 12 weeks, during which the patients had to make a total of three visits (0, 4th and 12th week). At their first visit, before administration of the study drug, the baseline lipid profile including total cholesterol (TC), LDL, high-density lipoprotein (HDL) and triglycerides (TGL) and Lp(a) values in serum, were recorded. During their second and third visit (4th and 12th week), the patients underwent general physical examination and were assessed for compliance to study medication and any adverse drug reactions. Blood samples were collected for lipid profile analysis and Lp(a) estimation.

Investigations

For the estimation of lipid parameters, about 5 ml of blood was drawn by venipuncture under aseptic conditions in a sterile vacutainer after a fasting period of 12 hrs overnight. Serum was immediately separated by centrifugation (10 mins at approximate 15,000 rpm) and stored at 2~4°C and analyzed for Lp(a) and lipid profile every 10 days. Determination of Lp(a) was done using an *in-vitro* latex reagent (Manufactured by Biolatex, Spain; Marketed by Diatek, Kolkata, West Bengal, India) by means of particle-enhanced turbidimetric immunoassay method.[22] TC, HDL and TGL in serum were measured by enzymatic methods using commercially available kits (Biosystems S.A. Barcelona). LDL was calculated by Friedewald's
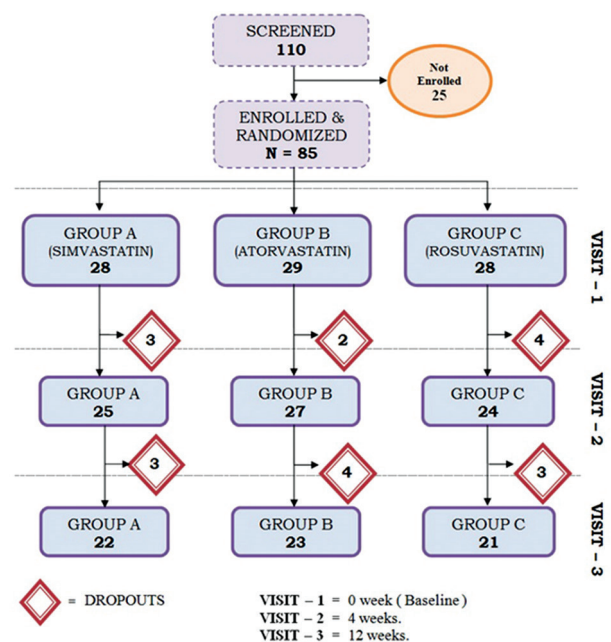

Figure 1: The movement of the patients in the three parallel groups of this study and the number of patients who were lost in follow-up at each visit.

formula. All laboratory investigations were carried out following Good Laboratory Practice, at the Department of Biochemistry in Annamalai University, Chidambaram, Tamil Nadu, India.

Statistical methods

The randomization of patients into the three parallel groups was done by an online computer algorithm. The sample size was approved by the Institutional Ethics Committee and was determined by the number of patients visiting the outpatient department for a master health check-up and who fulfilled the eligibility criteria of this study. Descriptive data are expressed as mean±standard deviation. One-way ANOVA was applied for analysis of all the lipid parameters in each group; significance $p < 0.001$. Duncan's Multiple Range Test was applied for analysis of Lp(a) levels among the three groups; significance $p < 0.05$. All statistical analysis was performed using SPSS version 17.0 (SPSS, Inc., Chicago, Illinois).

RESULTS

Of the 85 patients who were enrolled in the study, a total of 66 patients completed the 12 weeks statin therapy as per the study specifications. Data from patients who completed all the three study visits, 22 in Group A, 23 in Group B and 21 in Group C, were only taken up for statistical analysis.

The mean age of the three study groups was almost the same. The ratio of male to female patients in each group was not even. There were about 50% more males than females in each group. The average weight and body mass index of patients in each group at the start of the study was also quite similar for all the three groups. The lipid profile of all the three groups were also similar, except for TGL and Lp(a) values that showed a small variance of 10% between each other. Thus, all the three groups share similar physical and clinical conditions before the start of their respective therapy (Table 1).

After 12 weeks of therapy, atorvastatin was found to be most effective in reducing serum Lp(a) levels. There was an average of 18.73% ($p \leq 0.05$) reduction in serum Lp(a) levels from the baseline. This reduction was more significant in the first 4 weeks of atorvastatin therapy, with about 14.65% ($p \leq 0.05$) reduction from baseline. Group A patients, treated with simvastatin 20 mg/day also showed little reduction in the serum Lp(a) levels, but it was not significant. On the contrary, Group C patients, treated with rosuvastatin, showed an increase in serum Lp(a) levels throughout the study period. Though this percentage of increase is insignificant, it is noteworthy (Table 2).

Patients who received rosuvastatin 5 mg showed a reduction in the mean serum TC levels by 53.18% ($p \leq 0.001$) from baseline. Patients who received atorvastatin 10 mg and simvastatin 20 mg had a reduction in average serum TC by 45.40% ($p \leq 0.001$) and 34.80% ($p \leq 0.001$) respectively. Patients after atorvastatin therapy showed a significant reduction in average serum LDL levels from baseline after 4 weeks and 12 weeks by 65.47% ($p \leq 0.001$) and 88.87% ($p \leq 0.001$) respectively. Similar effect was produced by rosuvastatin on the LDL levels (Table 3).

Considering the mean serum HDL levels, the rosuvastatin group showed a substantial increase by 51.66% ($p \leq 0.001$) at 4 weeks of therapy. Whereas, this increase in percentage, fell back to 27.23% ($p \leq 0.001$) at the end of 12 weeks of treatment, the simvastatin and atorvastatin groups showed 38.21% ($p \leq 0.001$) and 45.10% ($p \leq 0.001$) increase in serum HDL levels respectively at the end of study period. TGL levels were reduced by all the three groups significantly. No adverse drug reactions were reported during or after this study.

DISCUSSION

CVD is recognized as a dreaded scourge in both developed and developing countries especially after studies established

Table 1: Baseline demographic characteristics and lipid profile of study patients.**

Criteria	Group A (simvstatin 20 mg/day)	Group B (atorvastatin 10 mg/day)	Group C (rosuvastatin 5 mg/day)
Age (years)	46.5±6.7	47.9±7.1	50.8±6.1
Male n (%)	13 (59.1)	15 (65.2)	12 (57.1)
Female n (%)	9 (40.9)	8 (34.8)	9 (42.9)
Weight (kg)	76.5±8.6	76.3±8.3	73.4±8.3
BMI (kg/m^2)	30.3±3.6	29.8±4.2	29.8±3.6
TC (mg/dl)	222.86±8.9	219.7±10.1	223.43±12.5
LDL (mg/dl)	143.68±8.9	144.04±11.3	145.62±10.2
HDL (mg/dl)	43.26±5.5	39.35±9.7	43.15±9.1
TGL (mg/dl)	179.59±8.4	181.52±11.9	173.29±22.1
Lp (a) (mg/dl)	35.77±5.7	39.17±6.2	38.24±5.4

**Values are expressed in mean±SD, BMI: Body mass index, TC: Total cholesterol, LDL: Low density lipoproteins, HDL: High density lipoproteins, TGL: Triglycerides, Lp(a): Lipoprotein (a)

Table 2: Variation in Lp(a) levels at 4 weeks and 12 weeks of treatment period across the three groups.**

Group	Lp (a) (mg/dl)		
	Visit 1 (baseline)	Visit 2 (4 weeks)	Visit 3 (12 weeks)
Group A (n=22) (simvstatin 20 mg/day)	35.77±5.78[a]	34.86±5.27[a] ↓ (2.55%)	34.63±6.26[a] ↓ (3.15%)
Group B (n=23) (atorvastatin 10 mg/day)	39.17±6.23[b]	33.43±4.73[a] ↓ (14.65%)	31.83±4.93[c] ↓ (18.73%)
Group C (n=21) (rosuvastatin 5 mg/day)	38.24±5.44[b]	39.29±5.7[b] ↑ (2.75%)	41.52±5.65[b] ↑ (8.58%)

**Values are expressed in mean (mg/dl) and ±SD with the percentage variation at 4 weeks and 12 weeks of treatment from baseline. Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT), DMRT: Duncan' multiple range test, SD: Standard deviation, Lp(a): Lipoprotein (a), SD: Standard devaiton

Table 3: Variations in lipid profile and serum Lp(a) levels at 4 weeks and 12 weeks of treatment period across the three groups.**

Lipid profile (mg/dl)	Mean±SD (% variation)					
	Group A (n=22) simvastatin 20 mg/d		Group B (n=23) atorvastatin 10 mg/d		Group C (n=21) rosuvastatin 5 mg/d	
	Visit 2 (4 weeks)	Visit 3 (12 weeks)	Visit 2 (4 weeks)	Visit 3 (12 weeks)	Visit 2 (4 weeks)	Visit 3 (12 weeks)
TC	195.27±7.8 ↓ (23.61%)#	182.23±7.1 ↓ (34.80%)#	186.17±9.5 ↓(29.26%)#	167.7±9.4 ↓(45.40%)#	182.52±10.7 ↓ (34.91%)#	161.14±9.4 ↓ (53.18%)#
LDL	106.27±8.4 ↓ (49.80%)#	93.59±7.8 ↓ (66.59%)#	94.83±7.8 ↓ (65.47%)#	83.26±7.2 ↓ (88.87%)#	90.14±5.1 ↓ (72.55%)#	80.62±6.1 ↓ (85.16%)#
HDL	58.31±9.5 ↑ (35.30%)#	59.6±9.9 ↑ (38.21%)#	60.65±9.2 ↑ (59.17%)#	55.23±8.9 ↑ (45.10%)#	63.85±9.6 ↑ (51.66%)#	53.38±8.1 ↑ (27.23%)#
TGL	153.45±10.6 ↓ (27.88%)#	145.18±6.9 ↓ (36.63%)#	153.48±9.7 ↓ (29.50%)#	146.04±9.8 ↓ (37.39%)#	142.67±18.9 ↓ (33.80%)#	135.71±17.4 ↓ (41.37%)#
Lp (a)	34.86±5.2 ↓ (2.55%)[ns]	34.64±6.3 ↓ (3.15%)[ns]	33.44±4.7 ↓(14.65%)#	31.83±4.9 ↓(18.73%)#	39.29±5.7 ↑ (2.75%)[ns]	41.52±5.7 ↑ (8.58%)[ns]

**Values are expressed in mean (mg/dl) and ±SD with the percentage variation at 4 weeks and 12 weeks of treatment from baseline. #Significance $p \leq 0.001$, compared to baseline, ns: Not significant ($p \geq 0.05$), TC: Total cholesterol, LDL: Low density lipoproteins, HDL: High density lipoproteins, TGL: Triglycerides, Lp(a): Lipoprotein (a), SD: Standard deviation

its role as the leading cause of mortality in India and International community. Although LDL-C has been highlighted as the primary lipid that causes atherosclerotic CVD,[3,4] the mystery shrouding CVD is not totally resolved. To trace, identify and scrutinize, other non-classical, independent risk factors of CVD, is still a challenge facing the medical professionals.

Lp(a), has been identified as one such independent risk factor for CVD. Owing to our poor knowledge of the metabolic paths of Lp(a), particularly with respect to the catabolism, pharmaceutical science has not yet developed drugs that are able to reduce elevated Lp(a) concentrations to the desired levels.[23] This comparative study of statins was primarily aimed at studying the effect of statins on Lp(a) levels. Although Lp(a) is structurally similar to an LDL moiety, we can't conclusively explain why it has a contradictory effect in case of rosuvastatin monotherapy, whereas it suffers a reduction impact in atorvastatin therapy. Gonbert et al. (2002) performed a large cohort study, which showed a significant decrease in the Lp(a) concentration, after a 6 weeks monotherapy of atorvastatin and simvastatin.[24] In this clinical study, the

12 weeks monotherapies of atorvastatin and simvastatin have shown a similar decrease in Lp(a) concentration. At the same time, rosuvastatin monotherapy has shown to elevate the Lp(a) levels substantially. This was on par with the findings of McKenney et al. (2007); who demonstrated that rosuvastatin monotherapy for 12 weeks significantly increases Lp(a) concentration, whereas when combined with niacin, it tends to reduce serum Lp(a) concentration.[14] Here too, though the results correlate, the foundations and methods of the two researches differ. McKenney et al. adapted rosuvastatin monotherapy to compare with combination therapies, while we made a comparative study of three statins only in monotherapy. However, the results of both studies show that rosuvastatin steadily increases the serum Lp(a) levels.

Of the three groups that underwent statin monotherapies in this study, it was evident that Atorvastatin reduced the Lp(a) levels significantly; in spite of the fact that rosuvastatin had an upper hand over rest of the lipid parameters. Further, Hernandez et al. (2011) demonstrated a similar effect in his randomized control trial, that a 12 weeks monotherapy of atorvastatin effectively reduced serum Lp(a) concentration.[25]

CONCLUSION

Lp(a) is a proven independent risk factor of CVD, yet it is not being screened for, in general population and LDL levels are still considered as the primary target for controlling CVD. Although there are many other lipid lowering therapies, like nicotinic acid, fibrates and bile acid sequestrates, statins have stood the test of time as the first line therapy for hypercholesterolemia. Combination therapies of statins can only supplement the use of statins but cannot supplant them. In developing countries like India, where pharmacoeconomics plays a major role in the choice of drugs, long-term statin monotherapy is preferred for a better prognosis in CVD patients and for a better prophylaxis in patients identified with a high risk of CVD. This study findings may be limited by its sample size owing to time and financial constraints. But still, this study clearly shows that atorvastatin monotherapy may be a better choice for patients with a high risk of CVD, when compared to other statin monotherapies.

ACKNOWLEDGMENT

REFERENCES

1. Gupta R, Guptha S, Sharma KK, Gupta A, Deedwania P. Regional variations in cardiovascular risk factors in India: India heart watch. World J Cardiol. 2012;4(4):112-20.
2. Braunwald E. Shattuck lecture – Cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med. 1997;337(19):1360-9.
3. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106(25):3143-421.
4. International Atherosclerosis Society (IAS) Harmonized Clinical. Guidelines on Prevention of Atherosclerotic Vascular Disease. Printed in March, 2003. Available at http://www.athero.cz/cze/odborna-doporuceni/ias_guidelines_ex_sum.pdf. Accessed 11 Sep 2014.
5. Sacks FM, Pfeffer MA, Moye' L, Brown LE, Hamm P, Cole TG, et al. Rationale and design of a secondary prevention trial of lowering normal plasma cholesterol levels after acute myocardial infarction: the Cholesterol and Recurrent Events trial. (CARE) Am J Cardiol. 1991;68(15):1436-46.
6. Dubé JB, Boffa MB, Hegele RA, Koschinsky ML. Lipoprotein(a): more interesting than ever after 50 years. Curr Opin Lipidol. 2012;23(2):133-40.
7. Luc G, Bard JM, Arveiler D, Ferrieres J, Evans A, Amouyel P, et al. Lipoprotein (a) as a predictor of coronary heart disease: the PRIME Study. Atherosclerosis. 2002;163(2):377-84.
8. Ariyo AA, Thach C, Tracy R, Cardiovascular Health Study Investigators. Lp(a) lipoprotein, vascular disease, and mortality in the elderly. N Engl J Med. 2003;349(22):2108-15.
9. Hernández C, Francisco G, Chacón P, Simó R. Lipoprotein(a) as a risk factor for cardiovascular mortality in type 2 diabetic patients: a 10-year follow-up study. Diabetes Care. 2005;28(4):931-3.
10. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation. 2000;102(10):1082-5.
11. Maher VM, Brown BG, Marcovina SM, Hillger LA, Zhao XQ, Albers JJ. Effects of lowering elevated LDL cholesterol on the cardiovascular risk of lipoprotein(a). JAMA. 1995;274(22):1771-4.
12. Marburger C, Hambrecht R, Niebauer J, Schoeppenthau M, Scheffler E, Hauer K, et al. Association between lipoprotein(a) and progression of coronary artery disease in middle-aged men. Am J Cardiol. 1994;73(11):742-6.
13. Insull W Jr, Basile JN, Vo AN, Jiang P, Thakkar R, Padley RJ. Efficacy and safety of combination therapy with niacin extended-release and simvastatin versus atorvastatin in patients with dyslipidemia: the SUPREME Study. J Clin Lipidol. 2009;3(2):109-18.
14. McKenney JM, Jones PH, Bays HE, Knopp RH, Kashyap ML, Ruoff GE, et al. Comparative effects on lipid levels of combination therapy with a statin and extended-release niacin or ezetimibe versus a statin alone (the COMPELL study). Atherosclerosis. 2007;192(2):432-7.
15. Fieseler HG, Armstrong VW, Wieland E, Thiery J, Schütz E, Walli AK, et al. Serum Lp(a) concentrations are unaffected by treatment with the HMG-CoA reductase inhibitor Pravastatin: results of a 2-year investigation. Clin Chim Acta. 1991;204(1-3):291-300.
16. Wanner C, Böhler J, Eckardt HG, Wieland H, Schollmeyer P. Effects of simvastatin on lipoprotein (a) and lipoprotein composition in patients with nephrotic syndrome. Clin Nephrol. 1994;41(3):138-43.
17. Umeda F, Watanabe J, Inoue K, Hisatomi A, Mimura K, Yamauchi T, et al. Effect of pravastatin on serum lipids, apolipoproteins and lipoprotein (a) in patients with non-insulin dependent diabetes mellitus. Endocrinol Jpn. 1992;39(1):45-50.
18. Hunninghake DB, Stein EA, Mellies MJ. Effects of one year of treatment with pravastatin, an HMG-CoA reductase inhibitor, on lipoprotein a. J Clin Pharmacol. 1993;33(6):574-80.
19. Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol. 1998;81(5):582-7.
20. Crouse JR 3rd, Frohlich J, Ose L, Mercuri M, Tobert JA. Effects of high doses of simvastatin and atorvastatin on high-density lipoprotein cholesterol and apolipoprotein A-I. Am J

Cardiol. 1999;83(10):1476-7, A7.

21. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, et al. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). Am J Cardiol. 2003;92(2):152-60.

22. Fujita S, Sano T, Katayama Y. Measurement of serum Lp(a) by COBAS MIRA using a latex immunoturbidimetric assay kit. J Clin Lab Anal. 1994;8(6):385-90.

23. Kolshinsky LM, Marcovina SM. Lipoprotein (a). In: Ballantyne CM, editor. Clinical Lipidology; A Companion to Braunwald's Heart Disease. Philadelphia: Elsevier; 2010:130-43.

24. Gonbert S, Malinsky S, Sposito AC, Laouenan H, Doucet C, Chapman MJ, et al. Atorvastatin lowers lipoprotein(a) but not apolipoprotein(a) fragment levels in hypercholesterolemic subjects at high cardiovascular risk. Atherosclerosis. 2002;164(2):305-11.

25. Hernández C, Francisco G, Ciudin A, Chacón P, Montoro B, Llaverias G, et al. Effect of atorvastatin on lipoprotein (a) and interleukin-10: a randomized placebo-controlled trial. Diabetes Metab. 2011;37(2):124-30.