Serum Levels of Lipoprotein(a) and Homocysteine in Patients on Hemodialysis Who Take Hydroxymethylglutaryl-CoA Reductase Inhibitors, Vitamin B6, and Folic Acid

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Introduction. High serum levels of lipoprotein(a) and homocysteine are risk factors of cardiovascular disease which are prevalent in patients on hemodialysis. Controversy exists about the effects of hydroxymethylglutaryl-CoA reductase inhibitors on serum lipoprotein(a) levels in patients on hemodialysis. Also, deficiency of some water soluble vitamins and administration of statins may raise serum levels of homocysteine in these patients. This study was designed to investigate serum levels of lipoprotein(a) and homocysteine in patients on hemodialysis who were taking a statin, vitamin B6, and folic acid.

Materials and Methods. We investigated on 152 patients with maintenance hemodialysis who were taking atorvastatin or lovastatin, vitamin B6, and folic acid for at least 6 months. Their serum levels were obtained to measure lipoprotein(a) and homocysteine levels, as well as triglyceride, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol.

Results. The mean serum values of total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol and triglyceride were significantly less than the maximum reference values ($P < .001$). The mean serum level of lipoprotein(a) was also less than the reference value ($P = .009$), but homocysteine level was 33% higher on average than the reference value ($P < .001$).

Conclusions. Our study demonstrated that in our patients on hemodialysis, the mean serum level of homocysteine was about 30% higher than the reference value although they were receiving vitamin B6 and folic acid. Hence, they were still exposed to the risk of cardiovascular disease.

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INTRODUCTION

Atherosclerosis is the major cause of mortality in patients with chronic kidney disease.[1] Lipoprotein(a) is a genetically determined low-density lipoprotein with a unique apolipoprotein A molecule that predisposes individuals to thrombotic complications of atherosclerosis and cardiovascular disease (CVD).[2] Hence, high serum lipoprotein(a) levels predict CVD in patients on hemodialysis.[3] There have been controversial studies about the effect of most lipid-lowering drugs on serum lipoprotein(a) levels. While some studies proposed several lipid-lowering drugs can reduce serum lipoprotein(a) levels, many failed to demonstrate this effect.[4-7]

Homocysteine is another predisposing factor of atherothrombosis through endothelial dysfunction,

enhancement of inflammation, and thrombophilic profile.[8] Serum level of homocysteine is increased in patients on hemodialysis and predisposes them to CVD.[9] Homocysteine also induces glomerular injury and sclerosis.[10] Deficiency of some water soluble vitamins, especially vitamin B6, vitamin B12, and folic acid may result in hyperhomocysteinemia. Thus, treatment with these vitamins can reduce serum homocysteine levels, and may lower the risk of CVD in patients on hemodialysis.[11,12]

On the other hand, some lipid-modifying and antihypertensive drugs have been shown to increase homocysteine serum levels.[13] Thus, a combination of vitamins and lipid-lowering drugs can have contradictory effects on homocysteine and lipoprotein(a) levels. The purpose of this study was to determine serum levels of lipoprotein(a) and homocysteine in patients on maintenance hemodialysis who were taking a hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitor, vitamin B6, and folic acid.

MATERIALS AND METHODS

In this cross-sectional study, we investigated patients on maintenance hemodialysis in 2 large referral hospitals. We selected 152 patients who were on antilipid therapy with either atorvastatin or lovastatin, both 20 mg/d; vitamin B6, 40 mg/d; and folic acid, 1 mg/d; for at least 6 months. They were undergoing dialysis 3 times a week for at least 1 year.

Their fasting blood samples which were being collected monthly for routine laboratory examinations were also analyzed for serum lipoprotein(a) and homocysteine levels. Serum level of lipoprotein(a) was assessed by enzyme-linked immunosorbent assay (Biopool US Inc, Ventura, California, USA) and homocysteine, by enzymatic cycling method (Diazyme, Dresden, Germany). The blood samples were also assessed for serum levels of triglyceride (enzymatic Gop-PAP method), total cholesterol (enzymatic-calorimetric CHOP-PAP method), high-density lipoprotein cholesterol (HDLC; direct enzymatic method), and low-density lipoprotein cholesterol (LDLC; calculated with Freidwald equation). All these biochemical assays were done using diagnostic kits made by Bioactiva Diagnostica (Homburg, Germany. Since the LDLC measurement with Friedwald equation is falsified when triglyceride is over 400 mg/dL, we excluded results of LDLC from the analyses in patient with this condition.

The 24-hour dietary recall was recorded 2 times after a 45-day interval in patients on hemodialysis to investigate variations in their food intake and to control diet-related confounding factors. The Food Processor II software (ESHA Research, Salem, Oregon, USA) was used to process macronutrients and micronutrients intakes based on the dietary reference intakes.[14] The independent *t* test and the paired *t* test were applied to analyze differences in variables between groups using the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA). Results of continuous variables were expressed as mean ± standard deviation. *P* values less than .05 were considered significant.

RESULTS

A total of 152 patients on hemodialysis, 91 men (59.9%) and 61 women (40.1%) were studied. The mean age of patients was 53.5 ± 14.0 years, ranged from 21 to 81 years (men, 53.6 ± 14.4 years and women, 53.2 ± 13.4 years). The mean serum values of lipoprotein(a), homocysteine, total cholesterol, LDLC, HDLC, and triglyceride in the patients and the reference values are shown in Table 1. All these values of serum lipid markers were

Table 1. Serum Levels of Lipoprotein(a), Homocysteine, and Lipids in Patients on Hemodialysis*

Serum levels	Men	Women	All Patients	Maximum RV	Mean Levels/RV, %	*P*
Lipoprotein(a), mg/dL	22.04 ± 2.15	29.72 ± 3.19	25.13 ± 1.83	30	83	.009
Homocysteine, mg/dL	19.75 ± 0.56	20.41 ± 0.75	20.01 ± 0.45	15	133	< .001
Total cholesterol, mg/dL	154.68 ± 3.93	152.83 ± 4.66	153.94 ± 3.00	200	77	< .001
LDLC, mg/dL†	86.02 ± 3.25	86.22 ± 3.54	86.10 ± 2.40	130	66	< .001
HDLC, mg/dL	41.54 ± 0.68	40.14 ± 0.85	40.98 ± 0.53	45	91	< .001
Triglyceride, mg/dL	148.01 ± 9.05	162.50 ± 10.78	153.82 ± 6.94	200	77	< .001

*Values are expressed as mean standard deviation. RV indicates reference values; LDLC, low-density lipoprotein cholesterol; HDLC, and high-density lipoprotein cholesterol.
†Three patients were excluded from analysis due to their high serum triglyceride levels (> 400 mg/dL).

Table 2. Daily Energy and Nutrient Intake of Patients on Hemodialysis

Energy and Nutrients	1st Evaluation	2nd Evaluation*	*P*	Dietary Reference Intakes[14]	
				Men	Women
Energy, kcal/d	1220.0 ± 190.5	1212.4 ± 252.3	.86	2700 to 3000	2000 to 2400
Protein, g/d	64.4 ± 8.9	64.8 ± 12.5	.84	56	46
Carbohydrate, g/d	137.0 ± 21.9	144.8 ± 26.0	.07	130	130
Fat, g/d	47.7 ± 13.7	46.2 ± 15.0	.62	…	…
Vitamin B6, mg/d	1.0 ± 0.2	1.0 ± 0.1	.68	1.3 to 1.7	1.3 to 1.5
Vitamin B12, µg/d	3.1 ± 1.2	3.1 ± 1.5	.95	2.4	2.4
Folic acid, µg/d	157.3 ± 33.0	152.2 ± 36.1	.41	400	400

*The 2nd evaluation was done after a 45 days interval. Ellipses indicate not determined.

significantly less than the maximum reference values ($P < .001$). The mean serum level of lipoprotein(a) was also less than the reference value ($P = .009$), but homocysteine level was 33% higher on average than the reference value ($P < .001$). The 24-hour dietary recall results are shown in Table 2. There were no significant variations between the two times in terms of energy and nutrients intakes.

DISCUSSION

Cardiovascular disease is the main cause of mortality in patients on hemodialysis.[1] Lipoprotein(a) and homocysteine are two independent risk factors of cardiovascular disease.[15] Our findings showed that serum lipoprotein(a) levels were 17% lower than the maximum normal level, on the average, in patients on hemodialysis who were taking a statin, folic acid, and vitamin B6. Serum homocysteine concentration, however, was 33% higher than normal in the studied patients.

Longenecker and colleagues showed contradictory results to our findings; serum lipoprotein(a) levels had increased in their patients on hemodialysis which could predispose them to CVD.[7] There are limited data on how diet and drugs affect serum lipoprotein(a) levels. Serum lipoprotein(a) levels are unaffected by lipid-lowering dietary treatment, and they increase even by dietary trans fatty acids.[16-18] Monte and Mezdour demonstrated that neither treatment with HMG-CoA reductase inhibitors, nor fibrate drugs resulted in significant lowering of lipoprotein(a) levels,[19] while Joy and colleagues and also Navarro and coworkers showed that atorvastatin could decrease serum lipoprotein(a) levels in patients on hemodialysis.[4,8] In our study, the mean serum level of total cholesterol, LDLC, HDLC, triglyceride, and lipoprotein(a) were significantly lower than reference values (Table 1), which confirms findings of the two latter studies. This may be, at least proportionally, because not only all our patients were taking atorvastatin or lovastatin for at least 6 month, but also their mean total energy intake were significantly less than the dietary reference intakes (Table 2).

Serum homocysteine levels are also high in patients on hemodialysis who are usually malnourished and deprived of enough vitamins.[9,19] Our study showed that the mean food intakes of vitamins B6 and folic acid were less than the dietary reference intakes (Table 2). Basically, these patients have poor appetite and are especially reluctant to take high-potassium vitamin-rich vegetables and fruits. The other problem is that water soluble vitamins can be excreted through dialysis membranes.[20] Our study showed even though these patients were taking vitamin B6 and folic acid supplements, their serum homocysteine levels were still high. This suggests that probably these therapeutic dosages had not been adequate to meet the need for these vitamins, or there are other unknown underlying metabolic reasons for high homocysteine levels that need further investigations.[21] For instance, Dierkes and colleagues showed that some lipid-lowering and antihypertensive drugs could increase serum levels of homocysteine.[1]

Overall, our main limitation of the study, which was the lack of a control group of patients on hemodialysis without statins and vitamins administration, precludes making a strong conclusion based on our findings. Therefore, it is appropriate to design a case-control study of patients on hemodialysis who are taking and not taking HMG-CoA reductase inhibitors and vitamins.

CONCLUSIONS

Our findings indicate that although patients

on hemodialysis, even if being on routine administration of HMG-CoA reductase inhibitors, vitamin B6, and folic acid supplements, may have serum homocysteine levels up to 30% more than normal, hence, exposing them to the risk of CVD. We need further investigations to determine whether this increase is due to administration of lipid-lowering drugs or other factors.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Dierkes J, Luley C, Westphal S. Effect of lipid-lowering and anti-hypertensive drugs on plasma homocysteine levels. Vasc Health Risk Manag. 2007;3:99-108.
2. Haffner S, Orchard T, Stein E, Schmidt D, LaBelle P. Effect of simvastatin on Lp(a) concentrations. Clin Cardiol. 1995;18:261-7.
3. 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;33:574-80.
4. Ikizler TA. Protein and energy intake in advanced chronic kidney disease: how much is too much? Semin Dial. 2007;20:5-11.
5. Joy MS, Dornbrook-Lavender KA, Chin H, Hogan SL, Denu-Ciocca C. Effects of atorvastatin on Lp(a) and lipoprotein profiles in hemodialysis patients. Ann Pharmacother. 2008;42:9-15.
6. Kaisar M, Isbel N, Johnson DW. Cardiovascular disease in patients with chronic kidney disease. A clinical review. Minerva Urol Nefrol. 2007;59:281-97.
7. Longenecker JC, Klag MJ, Marcovina SM, et al. High lipoprotein(a) levels and small apolipoprotein(a) size prospectively predict cardiovascular events in dialysis patients. J Am Soc Nephrol. 2005;16:1794-802.
8. Navarro JF, Mora C, Muros M, Garcia-Idoate G. Effects of atorvastatin on lipid profile and non-traditional cardiovascular risk factors in diabetic patients on hemodialysis. Nephron Clin Pract. 2003;95:c128-35.
9. Righetti M. Homocysteine-lowering vitamin B treatment decreases cardiovascular events in hemodialysis patients. Clin Chem Lab Med. 2007;45:1586-9.
10. Smolders B, Lemmens R, Thijs V. Lipoprotein (a) and stroke: a meta-analysis of observational studies. Stroke. 2007;38:1959-66.
11. Sunder-Plassmann G, Winkelmayer WC, Fodinger M. Therapeutic potential of total homocysteine-lowering drugs on cardiovascular disease. Expert Opin Investig Drugs. 2000;9:2637-51.
12. Wierzbicki AS. Homocysteine and cardiovascular disease: a review of the evidence. Diab Vasc Dis Res. 2007;4:143-50.
13. Yi F, Li PL. Mechanisms of homocysteine-induced glomerular injury and sclerosis. Am J Nephrol. 2008;28:254-64.
14. Food and Nutrition Board: Institute of Medicine. Dietary reference intakes *for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC:* National Academies Press; 2002.
15. Yilmaz FM, Yilmaz G, Duranay M, et al. Cardiovascular risk factors in hemodialysis and peritoneal dialysis patients. Scand J Clin Lab Invest. 2005;65:739-45.
16. Tsai YH, Park S, Snook JT. Interaction among Lp(a) phenotypes, Lp(a) concentrations and lipoprotein response to fat-modified diets. *J Nutr Biochem.* 1998;9:106-13.
17. Nestel P, Noakes M, Belling B, et al. Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet. J Lipid Res. 1992;33:1029-36.
18. Mensink RP, Zock PL, Katan MB, Hornstra G. Effect of dietary cis and trans fatty acids on serum lipoprotein[a] levels in humans. J Lipid Res. 1992;33:1493-501.
19. Monte G, Mezdour H. [The pharmacological effects of certain compounds on lipoprotein(a)]. Recenti Prog Med. 1993;84:855-63. Italian.
20. Heinz J, Domrose U, Westphal S, Luley C, Neumann KH, Dierkes J. Washout of water-soluble vitamins and of homocysteine during haemodialysis: effect of high-flux and low-flux dialyser membranes. Nephrology (Carlton). 2008;13:384-9.
21. van Guldener C, Stam F, Stehouwer CD. Homocysteine metabolism in renal failure. Kidney Int Suppl. 2001;78:S234-7.

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