ASSESSMENT OF SERUM LIPOPROTEIN(A) IN PATIENTS WITH END-STAGE RENAL DISEASE

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SUMMARY: Increased plasma lipoprotein(a) [Lp(a)] levels are strongly associated with premature atherosclerotic cardiovascular disease. The kidney is purported to play an important role in Lp(a) catabolism. However, race and genetic background to a large extent determine the Lp(a) concentrations in the general population. The aim of present study was to evaluate the effect of end-stage renal disease on serum Lp(a) in a group of Iranian population.

Sixty seven patients with end-stage renal disease treated by hemodialysis and 100 healthy controls were investigated. The lipids were measured by routine laboratory methods and Lp(a) assay was done by electroimmunodifusion.

The serum levels of Lp(a) in patients were significantly higher than controls (median 31 mg/dl versus 20 mg/dl, p=0.016). The Lp(a) in control group did not correlate with age, sex and lipids, but in patients it was found to correlate significantly with age (r=0.28, p=0.033), and cholesterol (r=0.35, p=0.008). In this study, 28% of controls and 54% of patients had serum Lp(a) above 30 mg/dl. This level is considered as cutoff point of high risk for atherosclerosis.

We concluded that end-stage renal disease could affect the serum lipids, Lp(a) and other lipoproteins towards atherogenic state in the Iranian population.

Key Words: Lipoprotein(a), end-stage renal disease.

INTRODUCTION

Lipoprotein(a) [Lp(a)] is a cholesterol-rich particle existing in human plasma (1). Many epidemiological and case-control studies have shown that, when present in high levels in the plasma, Lp(a) is recognized as an independent risk factor for premature atherosclerotic coronary heart disease (2, 3). However, the biological role and normal metabolism of this lipoprotein are not yet fully elucidated. Some studies have revealed an increase in plasma concentration of Lp(a) in patients with renal diseases (4, 5). Elevated plasma Lp(a) levels in renal patients had been associated with a frequency distribution of apolipoprotein(a) [apo(a)] isoforms similar to those found in general population. This indicates that elevated Lp(a) levels in these patients are not due to genetic origin (6). Therefore, it has been suggested that kidneys have an important role in Lp(a) catabolism (7). However, some studies did not confirm the role of kidney in the clearance of Lp(a) from plasma (8). Cardiovascular, cerebrovascular and peripheral vascular diseases are among the largest

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cause-specific reasons for morbidity and mortality in patients with end-stage renal disease (9). In addition, normal plasma levels of Lp(a) and also its atherogenic effects are strongly race and genetic dependent (10).

Since there was no documented data about the effect of renal failure on plasma Lp(a) level in Iran, the major aim of the present study was to evaluate the effect of end-stage renal disease on the plasma level of Lp(a) and other lipids in a group of Iranian population.

MATERIALS AND METHODS

Subjects and blood sampling

We studied 100 healthy controls and 67 patients. The patients included 57 males and 10 females with a mean age of 48 years, ranging from 12 to 79 years. They were in end-stage renal disease and were being treated by hemodialysis. The controls included 51 females, 49 males, with a mean age of 47 years, ranging from 15 to 75 years. They consisted of 50 normal subjects, that were the first relatives of patients, who proved to be healthy by health screening, and 50 blood donor volunteers. Nobody in controls had clinical or biochemical evidence of cardiovascular, hepatic or endocrine disorders, and all of them had serum creatinine concentrations of equal or lower than 1.2 mg/dl by the Jaffe method.

Blood samples were collected in the morning by venipuncture after an overnight fast and before hemodialysis in patients. Blood was allowed to clot at room temperature for about 1 h. Sera were separated from cells by centrifugation at 1500 X g for 10 min. From each serum, one 0.5 ml fraction was taken and immediately stored at -70°C for a maximum of six months until the Lp(a) assay.

Analysis of lipids, lipoproteins and nitrogen compounds

Serum total cholesterol (TC) and triglycerides (TG) concentrations were determined using routine enzymatic methods, cholesterol oxidase and glycerol oxidase respectively. High density lipoprotein-cholesterol (HDL-C) was determined by the same enzymatic method after precipitation of betalipoproteins by dextransulfate-$MgCl_2$. These methods were automated on Technicon RA-1000 Autoanalyser, and total coefficient of variations (C.V.) for TC and TG were less than 4% and 6% respectively. Low density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula, when the concentration of TG was below 400 mg/dl (11). Serum urea and creatinine were measured by automated urease and Jaffe methods respectively.

Lp(a) assay

The serum Lp(a) assay was done by electroimmunodifusion (12). Lp(a) standard and controls were from Dako (code no. X 0958, X 0962). Specific anti-Lp(a) antibody was prepared by immunization of rabbits with extracted Lp(a) from pooled Lp(a)-rich plasma and purified by affinity chromatography (13). The limit of detection for Lp(a) was considered to be 1 mg/dl, and an intra-assay C.V. of 5% (n=20) was obtained at 25 mg/dl.

Statistical methods

For statistical analysis SPSS software was used. Student's t-test was used for comparison of lipids, lipoproteins and nitrogen compounds in two groups. Mann-Whitney U-test was used to compare Lp(a) in patients and control groups. Pearson correlation test was used to evaluate correlation between Lp(a) and other variable. A p value of equal or less than 0.05 was considered to be significant.

RESULTS

The serum Lp(a) level in patients with a median of 30 mg/dl was significantly higher than control group with a median of 16 mg/dl (p=0.016). The other parameters of Lp(a) in two groups are summarized in Table 1. Lipids and lipoproteins in patients group were higher than controls. Table 2 summarizes the comparison of lipids, lipoproteins and nitrogen compounds in two groups.

Lp(a) did not correlate significantly with age, sex and lipid risk factor in control group, but in patient group it was correlate with age (r=0.28, p=0.033), TC (r=0.35, p=0.008), HDL-C (r=0.31, p=0.018), and LDL-C (r=0.32, p=0.023).

Twenty eight percent of control and 54% of patients had serum Lp(a) level above 30 mg/dl.

DISCUSSION

One of the most important causes of death in patients with renal disease is increased rate of atherosclerosis and cardiovascular disease (9). Increased plasma concentration of Lp(a) is considered to be a genetically determined, independent risk factor for premature atherosclerosis and coronary artery disease in general population (14, 15). Many studies, in various populations, have indicated that atherogenic lipids and lipoproteins, such as Lp(a), were increased in patients with various stage of renal disease (4, 5). The results of present study showed a significant elevation in plasma levels of TC,

Table 1: Comparison of plasma Lp(a) in patients with end-stage renal disease and control group.

	Case (n=67)	Control (n=100)	p value
Mean (mg/dl)	38	31	0.016*
Standard deviation	28	34	
Median (mg/dl)	31	20	
Minimum	1	2	
Maximum	105	150	

* According to Mann-Whitney U-test.

LDL-C and Lp(a) in patients with renal failure in comparison with healthy controls (Tables 1, 2). Similar to many other studies, we did not find a significant difference in plasma Lp(a) by sexes. Many similar observations were made by other authors. Sechi and coworkers studied 160 patients with early impairment of renal function (16). They found an increase in plasma Lp(a) levels in comparison with healthy controls. In another study, Sechi and colleagues, evaluated Lp(a) concentrations and apo(a) isoforms in a group of patients with moderate renal failure (17). They found an increased plasma Lp(a) concentrations in patients and a similar apo(a) isoform distribution between patients with renal disease and controls. Kimak and Solski, in a study on three groups of end-stage renal patients and a control group, have indicated that serum Lp(a) levels in patients treated by Chronic ambulatory peritoneal dialysis were significantly higher than patients treated by hemodialysis, but in both groups, it was significantly higher than controls (18). In

Table 2: Comparison of lipids, lipoproteins and nitrogen compounds in patients with end-stage renal disease and control group.

Variable (mg/dl)	Case Mean±SD	Control Mean±SD	p value
Urea	124±53	29±13	0.001
Creatinine	9.2±2.98	1±0.5	0.001
Cholesterol	204±41	149±40	0.008
Triglycerides	196±125	164±123	0.038
HDL-C*	30±7	47±11	0.033
LDL-C**	117±36	80±33	0.01

*High density lipoprotein-cholesterol

**Low density lipoprotein-cholesterol

apo(a) phenotype analysis, they found that, in patients groups, elevated Lp(a) levels were observed mainly in patients with high molecular weight phenotype. Apo(a) phenotype is an important determinant factor for plasma Lp(a) levels, and in general populations, high molecular weight phenotype coexist with low plasma Lp(a) levels (10). As have been shown by Sechi, Kimak and some others (17-19), in renal patients, elevated Lp(a) concentrations were observed mainly in patients with high molecular weight apo(a) phenotype. In the present study, we did not determine the apo(a) phenotype distribution in our population. In addition, there were no data available concerning the apo(a) frequency distribution in general population in Iran.

As reported by Misra *et al.* (20), and also have been indicated in the study of Kimak and Solski (18), treatment by hemodialysis may lower the plasma Lp(a) levels in end-stage renal patients. We did not know anything about the plasma levels of Lp(a) in our patients group before treatment by hemodialysis. They were on hemodialysis in average 2.3 years, and their plasma Lp(a) levels may reduced in this period of time.

Importantly, the present study showed that 54% of patients had Lp(a) values exceeding 30 mg/dl, but only 28% of controls showed this condition, and this difference was stronger in females (59% v.s. 23%). This level is considered as cutoff point of high risk for atherosclerotic disease, and also has been proposed a threshold value in the assessment of the risk of developing premature atherosclerosis (21). According to this cutoff point, the percentage of high risk in renal patients group, especially in females, was very high. It would be noteworthy that a limitation in our study was small sample size for patients group (n=67), specially females (n=17).

Finally we concluded that end-stage renal disease could affect lipids and lipoproteins profile towards an atherogenic state. In addition, relatively high percentage of end-stage renal patients are in high risk state for Lp(a), and this may be contribute to the increased mortality rate of these patients in Iran.

REFERENCES

1. Mbewu AD, Durington PN : Lipoprotein(a): Structure and possible involvement in thrombogenesis and atherogenesis. Atherosclerosis, 85:1-14, 1990.

2. Macrovina SM, Koschinsky ML : Lipoprotein(a) as a risk factor for coronary artery disease. Am J Cardiol, 82:57-66, 1998.

3. Orem A, Deger O, Kulan K, et al : Evaluation of lipoprotein(a) as a risk factor for coronary artery disease in the Turkish population. Clin Biochem, 28:171-173, 1995.

4. Kimak E, Solski J, Janicka L, Duma D, Zagojska M : Plasma lipoproteins in patients with chronic renal failure. Int Urol Nephrol, 29:597-601, 1997.

5. Greiber S, Wanner C : Lipoprotein(a) in nephrotic syndrome and end-stage renal disease. Miner Electrol Metab, 23:161-165, 1997.

6. Dieplinger H, Lackner C, Kronenberg F, et al : Elevated plasma concentrations of lipoprotein(a) in patients with end-stage renal disease are not related to the size polymorphism of apolipoprotein(a). J Clin Invest, 91:397-401, 1993.

7. Kronenberg F, Trenkwalder E, Lingenhel A, et al : Renovascular arteriovenous in lipoprotein(a) plasma concentrations suggest removal of Lp(a) from the renal circulation. J Lipid Res, 38:1755-1763, 1997.

8. Misra M, Reaveley DA, Cooper C, et al : Mechanism for elevated plasma lipoprotein(a) concentrations in patients on dialysis: turnover studies. Adv Perit Dial, 14:223-227, 1998.

9. Koda Y, Nishi S, Suzuki M, Hirasawa Y : Lipoprotein(a) is a predictor for cardiovascular mortality of hemodialysis patients. Kidney Int Suppl, 71:251-253, 1999.

10. Kim JQ, Song JH : High allele frequency of apolipoprotein(a) phenotype Lps4 is associated with low serum Lp(a) concentrations in Korean. Clin Biochem, 27:57-62, 1994.

11. Friedewald WT, Levy RI, Fredrickson DS : Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of centrifuge. Clin Chem, 18:449-502, 1972.

12. Winfried M, Werner G : Quantification of human serum lipoprotein(a): zone immunoelectrophoresis assay, a new sensitive method as compared to electroimmuno assay. Clin Chimi Acta, 134:265-279, 1983.

13. Abe A, Maeda S, Makino K, et al : Enzyme-linked immunosorbent assay of lipoprotein(a) in serum and cord blood. Clin Chimi Acta, 28:171-173, 1995.

14. Eric B, Carla C, Jingping L, et al : Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. J Clin Invest, 90:52-60, 1992.

15. Sankamp M, Funke H, Schlete H, et al : Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. Clin Chem, 36:20-23, 1990.

16. Sechi LA, Zingaro L, Carli S, et al : Increased serum lipoprotein(a) levels in patients with early renal failure. Ann Inter Med, 129:457-461, 1998.

17. Sechi LA, Zingaro L, Catena C, et al : Lipoprotein(a) and apolipoprotein(a) isoforms and protenuria in patients with moderate renal failure. Kidney Int, 56:1049-1057, 1999.

18. Kimak E, Solski J : Serum lipoprotein(a) concentrations and apolipoprotein(a) phenotypes in hemodialysis, chronic ambulatory peritoneal dialysis and post-transplant patients. Ren Fail, 24:187-195, 2002.

19. Kim SB, Yang WS, Kang ES, Min WK, Park JS : Lipoprotein(a) and apolipoprotein(a) phenotypes in patients with end-stage renal disease. Perit Dial Int, 17:236-242, 1997.

20. Misra M, Webb AT, Reaveley DA, et al : The effect of change of renal replacement therapy on serum lipoprotein(a) concentration. Adv Perit Dial, 13:168-173, 1997.

21. Angelo M, Scanu MD : Lipoprotein(a): A genetic risk factor for premature coronary heart disease. JAMA 267:3326-3329, 1992.

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