CLOSE RELATIONSHIP OF SERUM LIPOPROTEIN(A) WITH ULTRASONOGRAPHICALLY DETERMINED EARLY ATHEROSCLEROTIC CHANGES IN THE CAROTID AND FEMORAL ARTERIES IN END-STAGE RENAL FAILURE PATIENTS UNDERGOING HEMODIALYSIS

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SUMMARY: Lp(a) is recognized as an independent risk factor for premature atherosclerotic coronary heart disease. In renal failure, studies revealed an increase in plasma concentration of Lp(a), present study aimed to evaluate the effects of plasma Lp(a) levels on early structural atherosclerotic vascular changes in a group of end-stage renal failure patients under regular hemodialysis.

Sixty one unselected patients with end-stage renal disease (ESRD), undergoing maintenance hemodialysis treatment between September 2002 and December 2003 were examined, serum lipoprotein(a) was measured, using B-mode ultrasonography carotid-intimae-media thickness (IMT) measured as well as carotid-femoral plaques (plaque score) were determined.

Total patients (23 females, 38 males) consist 50 non-diabetic hemodialysis patients (20 females, 30 males), and 11 diabetic hemodialysis patients (3 females, 8 males). Mean±SD of Lp(a) of total patients were 58.5±19 mg/dl, for diabetic group were 62±12.3 mg/dl and for non-diabetic group were 57.7±20 mg/dl. In this study there were, more thickening of intimae-media complex in diabetic group, positive association of plaque score with ages and DM, positive correlation of carotid-IMT with carotid-femoral plaque score, positive correlation of serum Lp(a) with carotid-IMT and carotid-femoral plaque score. No significant difference of Lp(a) between diabetic and non-diabetic HD patients was found.

Diabetic hemodialysis patients had more accelerated atherosclerosis, lipoprotein(a) as a non-traditional factor in progression of atherosclerosis, can have a role in acceleration of rapid progressive atherosclerosis seen in these patients.

Key Words: Plaque score, hemodialysis, intimae-media thickness, diabetes mellitus.

INTRODUCTION

Lipoprotein(a) [Lp(a)] is a cholesterol-rich particle existing in human plasma, was first described by Berg in 1963 (1, 2). Many epidemiological and case-control studies have shown that when present in high levels of plasma, Lp(a) is recognized as an independent risk factor for premature atherosclerotic coronary heart disease (1-3). The exact mechanism by which Lp(a) is a cardiovascular risk is unknown, however, both proatherogenic and prothrombogenic effects have been hypothesized, but the biological role and normal metabolism of this lipoprotein are not fully

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elucidated (1-3). In renal failure, studies revealed an increase in plasma concentration of Lp(a) (5, 6). Elevated plasma Lp(a) levels in chronic renal failure patients have 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, 7), therefore, it has been suggested that kidneys have an important role in Lp(a) metabolism, decrease of Lp(a) catabolism or increase of liver production (7–11). In fact, with beginning of chronic renal failure and as glomerular filtration rate (GFR) reach below 70 ml/min, Lp(a) starts to increase (11–13), and dialysis procedure by itself does not seem to able to decrease Lp(a) levels (5–8, 12). Irrespective of pathophysiological mechanisms involved, increased Lp(a) levels could be a contributing factor in the increased incidence of atherosclerotic disease observed in hemodialysis patients (12–18). The early stages of atherosclerosis are associated with changes in arterial structure. Subtle structural changes, such as thickening of arterial intimae-media complex, occur early in the atherosclerotic disease process (15–18). Using B-mode ultrasonography for assessing early arteriosclerosis is safe and non-invasive to study superficial vascular districts, such as the carotid and femoral arteries (15–19). Therefore, ultrasonic evaluation of carotid artery for intimae-media thickness (IMT) and plaques can identify patients at risk for cardiovascular disease (15–18). Carotid arteries are privileged area for studying the progression of atherosclerotic lesions from onset to fully developed plaque. Carotid-IMT measurements are strongly related to the extent of atherosclerosis in other vascular districts too (15–18). As many known and conventional risk factors have been shown to be significantly associated with increased arterial wall thickness, consistent with their accepted role in atherogenesis, much less is known, however, about the effects of Lp(a) on IMT in patients under regular hemodialysis (1, 18). Therefore, the aim of present study was to examine the effects of plasma Lp(a) levels on early structural atherosclerotic vascular changes in a group of end-stage renal failure patients under regular hemodialysis.

MATERIALS AND METHODS

This is a cross-sectional study, was done on 61 unselected patients with end-stage renal disease (ESRD), undergoing maintenance hemodialysis treatment between September 2002 and December 2003. On patients selection exclusion criteria were cigarette smoking, body mass index (BMI) more than 25, anti lipid drug taking, recent MI and vascular diseases as well as active or chronic infection and inclusion criterion was hypertension. For all subjects lipoprotein(a) measured by enzyme linked immunosorbent assay (ELISA) by Immuno-Biological laboratories (IBL) kit, carotid sonography was done by a single sonologist unaware of history or lab data of patients, using a Honda-Hs-2000 Sonography and 7.5 MHZ linear probe, IMTs in mm were measured, the procedure was done at the end of diastolic phase, the sites of measurements were at the distal common carotid artery, area of bifurcation and at the first proximal internal carotid artery, IMT was measured at the plaque free areas, for examination, subjects were in supine position with neck hyperextension and rotation of head for facilitation of procedure performing. Carotid was evaluated in axial longitude. By sonography, the carotid artery is found to have three different echoes, Intimae, is as an echogen layer line, media-hypoecho, and adventitia is echogen. Intimae-media thickness was defined as the distance from leading edge of lumen-intimae interface of the far wall to the leading edge of the media-adventitia interface of the far wall. IMT more than 0.8 mm was considered abnormal, for statistical analysis we measured the mean of right and left carotid-IMT. Sonography for plaque was done at the right and left of carotid and femoral arteries and scored from 0 (no plaque) to 4 (plaque presence at all four sites) regardless of the number and size of the plaques in each site, plaque occurrence in each site, scored one point, plaques divided into 3 groups: soft, calcified and mixed, plaques were visualized as an echogen or hypoecho protrusion into the vessel lumen. Soft plaques are hypoecho, calcified are echogen and had shadow. Mixed plaques have heterogen echo, plaques were considered as a local intimal thickness more than 1 mm for plaque measurement the largest longitude was considered. For statistical analysis descriptive data are expressed as mean±SD, comparison between groups was evaluated by using chi-square (χ^2 test), Kruskal-Wallis test, Mann-Whitney U test and Fisher's exact test. For correlations Spearman test, partial correlation test with adjustment for age, Phi-Cramer's V test and

Table 1: Mean±SD, minimum and maximum of data.

		Age (year)	DHT* (month)	IMT (mm)	Lp(a) (mg/dl)
Total patients n=61	Mean±SD	46.5 ± 16	32 ± 31	1.06 ± 0.3	58.5 ± 19
	Min	15	2	0.50	25
	Max	78	108	1.70	154
Diabetic group n=11	Mean±SD	57 ± 16	22.6 ± 22.4	1.3 ± 0.3	62 ± 12.3
	Min	27	3	0.80	40
	Max	78	60	1.70	85
Non-diabetic group n=50	Mean±SD	47.8 ± 16	34 ± 33	1 ± 0.25	57.7 ± 20
	Min	15	2	0.50	25
	Max	78	108	1.60	154

*Duration of hemodialysis treatment.

Eta test were used. All statistical analyses were performed by using the statistical analysis system (SPSS version 11.00), statistical significance was inferred at a p<0.05.

RESULTS

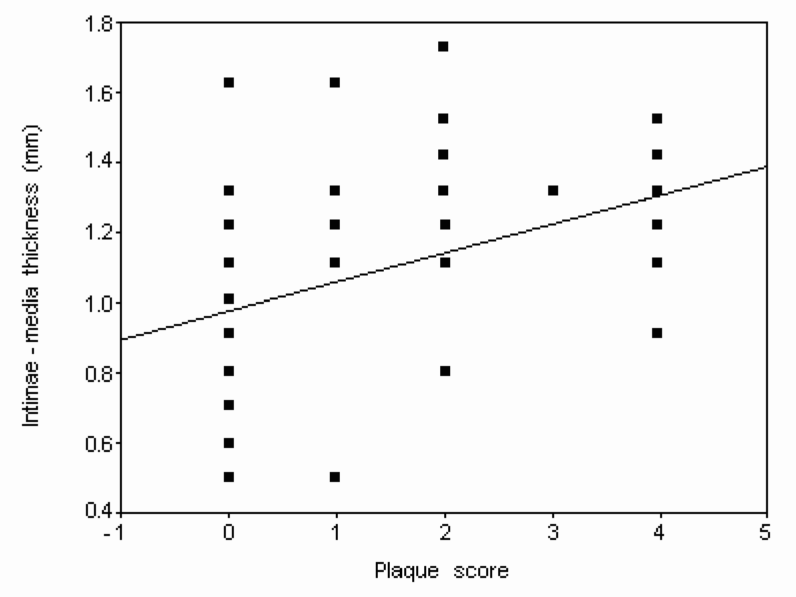
Total patients (23 females, 38 males), consist 50 non-diabetic hemodialysis patients (20 females, 30 males), and 11 diabetic hemodialysis patients (3 females, 8 males). Mean ± SD of ages of subjects were 46.5 ± 16 years. Mean ± SD of length of the time patients had been on hemodialysis were 32 ± 31 months. Mean ± SD of Lp(a) of total patients was 58.5 ± 19 mg/dl. Mean ± SD of Lp(a) of diabetic group was 62 ± 12.3 mg/dl and for non-diabetic group was 57.7 ± 20 mg/dl (Table 1). Table 2 shows the frequency distribution of plaque score in total patients consisted of diabetic and non-diabetic groups (all of the plaques were calcified) and all of the hemodialysis patients were hypertensive from stage one to three. In this study there was no significant difference of ages, and duration of hemodialysis between males and females (p>0.05) (Mann-Whitney U test), no significant difference of DM between two sexes (Fisher's exact test) (p>0.05), no significant difference of duration of hemodialysis and age of the patients between diabetic and non-diabetic

Table 2 : Frequency distribution of plaque scores of carotid and femoral arteries.

	Plaque score	Frequency	Percent
Total patients n = 61	0	33	54.1
	1	5	80.2
	2	12	19.7
	3	2	3.3
	4	9	14.8
Group 2 n = 11	0	1	9.1
	1	1	9.1
	2	6	54.5
	3	0	0.0
	4	3	27.3
Group 3 n = 50	0	32	64.0
	1	4	8.0
	2	6	12.0
	3	2	4.0
	4	6	12.0

Group 2: Diabetic HD patients
Group 3: Non-diabetic HD patients

Figure 1 : Significant positive correlation of carotid-femoral-plaque score with carotid-IMT (r=0.306, p=0.009) (partial correlation test after adjustment for age).

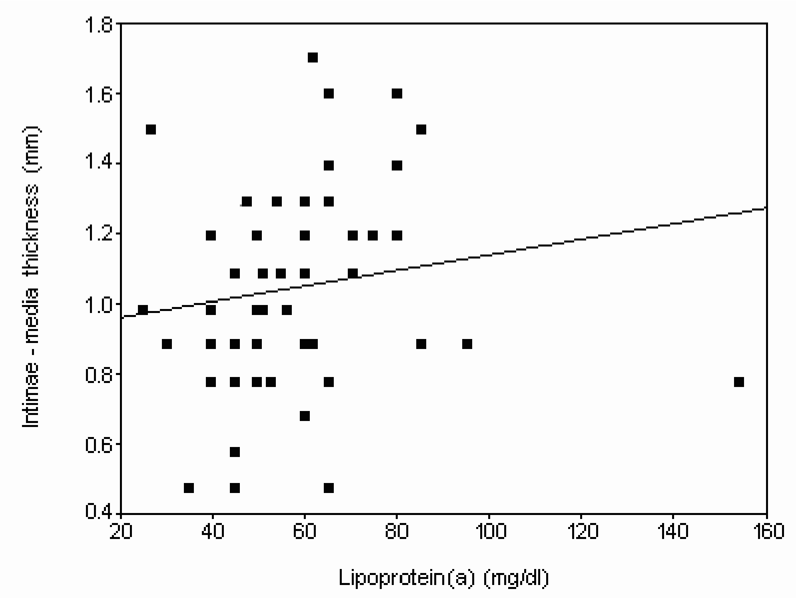


group existed (p>0.05) (Mann-Whitney U test). There was a significant difference of carotid-IMT between diabetic and non-diabetic group (1.3 ± 0.3 versus 1 ± 0.25 mm respectively) (p<0.05) (Eta test). No significant difference of duration of hemodialysis treatment as well as serum lipoprotein(a) and ages of patients between diabetic and non-diabetic group (p>0.05) (Mann-Whitney U test) existed, about plaque score, positive correlation of plaque scores with ages of the patients (p=0.003), was demonstrated (Kruskal-Wallis test). Significantly positive correlation between plaque scores and diabetes mellitus was observed (p=0.004) (χ^2 test). Significantly positive correlation between plaque scores and length of the time patients had been on hemodialysis (r= 0.239, p=0.033) as well as significantly positive correlation between carotid-Femoral plaques (plaque scores) and carotid-IMT was demonstrated (r=0.306, p=0.009) (partial correlation test after adjustment for age) (Figure 1). Statistical analysis on carotid-IMT and partial correlation test (after adjustment for ages) showed no positive correlation between IMT and duration of hemodialysis (p>0.05). Statistical analysis on Lp(a) showed significant positive correlation between carotid-IMT and lipoprotein(a) (r=0.330 p=0.009) (Figure 2) (Spearman test), also there was a significantly positive correlation between plaque scores and lipoprotein(a) (r=0.205, p=0.051) (Figure 3) (partial correlation test after adjustment for age). Finally after divided of patients into diabetic and non-diabetic for analysis of the correlation mentioned above, in 2 groups respectively, no other important data were found.

DISCUSSION

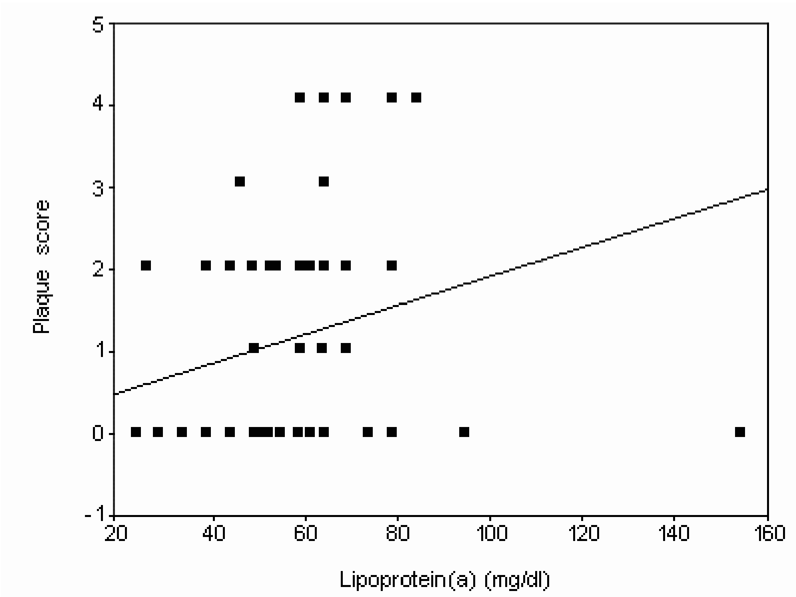
In this study there were, more thickening of Intimae-media complex in diabetic group, positive association of plaque score with ages and DM, positive correlation of carotid-IMT with carotid-femoral plaque score, positive correlation of serum Lp(a) with carotid-IMT and carotid-femoral plaque score, no significant difference of Lp(a) between diabetic and non-diabetic HD patients too. Pascasio *et al.* observed a large number of vascular plaques in uremia patients, he concluded that the process of advanced atherosclerosis might have started with the beginning of renal failure and suggested that hemodialysis treatment may not be a potential factor to accelerate atherosclerosis. Finally he concluded that the progression of atherosclerosis might be related to atherogenic factors operative before regular dialysis (19). Damjanovic *et al.* evaluated IMT of 45 dialysis patients and found higher

Figure 2: Significantly positive correlation of carotid-IMT with Lp(a) (r=0.330, p=0.009) (Spearman test).



mean carotid-IMT in HD patients than in control group, he showed positive correlation of IMT with certain risk factors for atherosclerosis (age, duration of dialysis and lipid parameters) (20). Correlation of IMT with ages and duration of hemodialysis in HD patients was evaluated by Shoji and Hojs *et al.* No clear relationship of IMT with duration of hemodialysis was found in their studies (21, 22). Hojs also in his study (on 28 HD patients) observed, age was the only significant determinant of number of plaques. He concluded that hemodialysis patients had

Figure 3: Significantly positive correlation between carotid-femoral-plaque score and Lp(a) (r=0.205, p=0.051) (partial correlation test after adjustment for age).



advanced atherosclerosis in the carotid arteries compared with normal subjects (22). Moreover, in his recent study, Hojs showed no difference in plaque occurrence between 28 hemodialysis patients and 28 ESRD patients prior to hemodialysis (23). In a study on 24 dialysis patients Savage *et al.* noted more prevalence of plaque in carotid and femoral artery. Also this study showed the relationship between femoral artery plaque and age, and he reported the correlation of age with IMT of carotid artery of HD patients (24). Moreover in a recent study by Kato *et al.*, there was a significant correlation of IMT with age on 219 HD patients (25). Sramek *et al.* on 142 asymptomatic men found no increased IMT in the carotid or femoral artery at high levels of Lp(a), he concluded that Lp(a) levels are not associated with early atherosclerotic vessel wall changes in the carotid or femoral arteries (26). Dentil *et al.* in a study on 100 elderly subjects (aged 78.5±0.6), showed no association between carotid-IMT and Lp(a), he concluded, that Lp(a) was unrelated to the severity of extra cranial vessels atherosclerosis (27), while Baldassarre *et al.* in a study on 100 type 2 hypercholesterolemic patients, showed higher values of carotid-IMT in hypercholesterolemic patients with plasma Lp(a) levels >30 mg/dl than in those with lower levels, he concluded that elevated plasma levels of Lp(a) can be considered an additional independent factor associated with thickening of carotid artery in patients with severe hypercholesterolemia but not in those with moderate hypercholesterolemia or normocholestrolemic subjects (28). Finally Raitakari *et al.* on 241 healthy subjects suggested no association between IMT and Lp(a), but significantly positive correlation with total cholesterol, LDL-c, LDL/HDL ratio, age, and Tg was found (1). In the present study, a significant association between serum lipoprotein(a) and carotid-IMT and carotid-femoral plaques was found, as the extraordinary high mortality in end-stage-renal disease (ESRD) patients under hemodialysis is due to cardiovascular disease, there is interest to non-traditional atherosclerotic cardiovascular disease risk factors that are prevalent in ESRD, such as lipoprotein(a), needs to more attention, because of its effect to acceleration of rapid progressive atherosclerosis seen in HD patients.

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