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Lipids in Association with Leptin in Maintenance Hemodialysis Patients

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To consider the association of serum leptin with dyslipidemia in hemodialysis patients. For maintenance hemodialysis patients levels of serum pre and post dialysis blood urea nitrogen, lipid profiles and serum Leptin were measured. For the adequacy of hemodialysis the urea reduction rate was calculated. A significant difference of serum leptin between males and females of diabetics with more values in female dialysis patients was seen. In total patients a near significant and inverse correlation of serum leptin with serum LDL was seen. In male hemodialysis group a significant positive correlation of serum leptin with duration of hemodialysis and with the ages of the patients were seen, in this group also a near significant positive correlation of serum leptin with serum triglyceride levels was seen too. In total patients there was a near significant positive correlation of serum leptin with serum cholesterol of patients who had a cholesterol levels of more than 100 mg dL^{-1}. Present data supports the hypothesis that in patients on hemodialysis, the association of leptin with cholesterol and triglyceride levels could show the positive effects of leptin on nutrition in hemodialysis patients which is in contrast to normal population.

Key words: Leptin, hemodialysis, lipids, malnutrition, URR, hemodialysis adequacy, cholesterol, triglyceride

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INTRODUCTION

Malnutrition due to poor food intake is a common clinical problem in patients with End-Stage Renal Disease (ESRD) (Aparicio *et al.*, 1999; Bergström, 1995) and is associated with an increase in morbidity and mortality in patients on haemodialysis treatment (Bergström, 1995). In dialysis patients, malnutrition is an independent factor causing morbidity and mortality. Both inadequate alimentation and metabolic alterations, which involve nitrogen and energy metabolism, contribute to malnutrition (Aparicio *et al.*, 1999; Bergström, 1995 Bergström, 1995). About 40% of patients undergoing maintenance dialysis suffer from varying degrees of protein-energy malnutrition. This is a problem of substantial importance because many measures of nutritional status correlate with the risk of morbidity and mortality (Fox *et al.*, 2004; Bossola *et al.*, 2004). The nutrient intake of patients receiving maintenance dialysis also is often inadequate and several lines of evidence suggest that toxins that accumulate with renal failure suppress appetite and contribute to nutritional decline once patients are on maintenance dialysis (Fox *et al.*, 2004; Bossola *et al.*, 2004). Serum leptin levels are elevated in patients with chronic renal insufficiency and end-stage renal disease and experimental evidence suggests a possible role for leptin in the development of protein-energy malnutrition in this population (Bossola *et al.*, 2004; Norton, 2002). Release of leptin from adipocytes may be stimulated by cytokines mediating the inflammatory response, which is frequently pronounced in patients with end-stage renal disease receiving hemodialysis (Norton, 2002; Mehrotra and Kopple, 2002). Leptin is also reported to have effects in peripheral tissues that are independent of its central effects on food intake and body weight. In a study, the acute effects of a single dose of recombinant mouse leptin on lipid metabolism in lean and gold thioglucose-injected obese mice were examined, changes were measured 2 h after leptin injection. Leptin in the maintenance of steady-state energy stores by decreasing lipid synthesis and increasing fat mobilization, occurring as a result of increased fatty acid oxidation (Bryson *et al.*, 1999). Therefore independent of its effects on food intake leptin has also been shown to have effects on lipid metabolism that are independent of its effects on food intake and that may occur before any loss of body weight (Bryson *et al.*, 1999). In end-stage renal failure, dyslipidemia is linked to risk of cardiovascular disease (Nasri and Baradaran, 2004). Increased concentrations of triacylglycerol-rich, Very Low Density Lipoproteins (VLDL) and decreased concentrations of High Density Lipoproteins (HDL) are usual, whilst total cholesterol and Low Density Lipoprotein (LDL) concentrations are not increased (Nasri and Baradaran, 2004; Gillett *et al.*, 2004). In the general population, hypercholesterolemia is a known risk factor for cardiovascular morbidity and mortality. Among lipid components, increased serum levels of Low-Density Liproprotein (LDL) and non High Density Lipoprotein (HDL) cholesterol appear to have the strongest predictive value for poor cardiovascular outcome, whereas HDL-cholesterol is generally considered protective in the general population (Kalantar-Zadeh *et al.*, 2003). In hemodialysis patients hypercholesterolemia appear to be a protective feature that are associated with a greater survival among dialysis patients (Kalantar-Zadeh *et al.*, 2003). This finding is in contrast to the well-known association between over-nutrition and poor outcome in the general population (Kalantar-Zadeh *et al.*, 2003; 2005). The association between under-nutrition and adverse cardiovascular outcome in dialysis patients, which stands in contrast to that seen in non-ESRD individuals, has been referred to as reverse epidemiology (Kalantar-Zadeh *et al.*, 2003; 2005). In this regard we aimed to conduct a study to find a possible role for leptin on dyslipidemia specially on cholesterol and serum LDL and triglyceride levels in hemodialysis patients to find whether leptin has a role on the regulation of lipids in hemodialysis. In fact association of leptin with malnutrition and its possible role as a cardiovascular risk (Aguilera *et al.*, 2002) and also the inverse association of total cholesterol levels with mortality in hemodialysis patients which is likely due to the cholesterol-lowering effect of malnutrition may show the probable role of leptin in the synthesis of the lipids. We therefore sought to consider the correlation of serum leptin with dyslipidemia in hemodialysis patients containing diabetic and non-diabetic populations.

MATERIALS AND METHODS

Patients: This cross-sectional study was conducted on patients with End-Stage Renal Disease (ESRD), who were undergoing maintenance hemodialysis treatment with acetate basis dialysate and polysulfone membranes. According to the severity of secondary hyperparathyroidism, each patient being treated for secondary hyperparathyroidism was given oral active vitamin D3 (Rocaltrol), calcium carbonate and Rena-Gel capsules at various doses. According to the severity of anemia, patients were under IV iron therapy with Iron sucrose (venofer) at various doses after each dialysis session, all patients were under treatments of 6 mg folic acid daily, 500 mg L-Carnitine daily, oral Vitamin

B-complex tablet daily and also 2000U IV Eprex (recombinant Human Erythropoietin (rHuEPO) equal for each patient after each dialysis session routinely. Exclusion criteria were active or chronic infection and using NSAID or ACE inhibitor drugs. The study was done in hemodialysis section of Hajar Medical, Educational and Therapeutic Center of Shahrekord University of Medical Sciences in Shahrekord of Iran.

Laboratory methods: After 12 h fasting, levels of serum pre and post dialysis Blood Urea Nitrogen(BUN) and also lipid profile containing serum Triglyceride (TG), Cholesterol (Chol) and high density lipoprotein (HDL-C) level were measured using standard kits. Serum Leptin (normal range of values for males is 3.84±1.79 and for females is 7.36±3.73 ng mL^{-1} was measured by enzyme-linked immunosorbent assay (ELISA) method using DRG of Germany. For the adequacy of hemodialysis the Urea Reduction Rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data (Boag, 1994). The Body Mass Index (BMI) was calculated using weight and height (kg m^{-2}) (http://www.halls.md/body-mass-index/av.htm). Serum LDL-C was calculated using friedewal's formula (Friedewald *et al.*, 1972). Excluasion criteria were the presence of active or chronic infection during the last months before the study. Duration and dosages of hemodialysis treatment were calculated from the patients' records. The duration of each hemodialysis session was 4 h.

Statistical analysis: Results are expressed as the Mean±SD and median values. Comparison between the groups was done using Student's t-test. Statistical correlations were assessed using partial correlation test. For some correlations the logarithm of data were used. Statistical analysis was performed on total Hemodialysis (HD), females, males, diabetics and non diabetics populations separately. All statistical analysis were performed using SPSS (version 11.5.00). Statistical significance was determined at a p<0.05.

RESULTS

Results of the total patients (N = 36; F = 14 M = 22), 26 were non-diabetic HD patients (F = 10, M = 16) and 10 were diabetic HD patients (F = 4, M = 6). Table 1 shows the patients' data of total, non diabetic and diabetic dialysis patients. The mean patient age was 46±16 years. The mean length of the time patients had received hemodialysis was 30±36 (median: 17.5) months. The mean serum leptin was 7±9.2 (median: 4.2) ng mL^{-1}. The mean serum leptin values within the diabetic and

Table 1: Patients data

| Total patients n = 36		Minimum	Maximum	Mean±SD	Median
Age	years	16	80	46±16	43
DH*	months	2	156	30±36	17.5
Dialysis					
dose	sessions	18	1584	285±396	144
URR	%	39	76	59±9	57.5
Leptin	mg dL^{-1}	0.1	52	7±9.1	4.2
Chol	mg dL^{-1}	59	211	117±39	115.5
TG	mg dL^{-1}	29	461	130±95	94
LDL	mg dL^{-1}	12	122	61.6±22	36.5
HDL	mg dL^{-1}	20	70	38±11	60
Non diabetics n = 26					
Age	years	16	80	43.6±16.5	41.5
DH*	months	2	156	37±41	20.5
Dialysis					
dose	sessions	18	1584	349±451	154.5
URR	%	50	76	60.6±7.78	60
Leptin	mg dL^{-1}	0.1	52	6.8±10	3.45
Chol	mg dL^{-1}	59	171	111 ±32	115
TG	mg dL^{-1}	61	461	128±84	97
LDL	mg dL^{-1}	12	99	62 ±20	36
HDL	mg dL^{-1}	25	70	39 ±11	60
Diabetics n = 10					
Age	years	27	75	51±16	55
DH*	months	6	24	15±6	12
Dialysis					
dose	sessions	54	216	127±55	99
URR	%	39	75	54±10	54
Leptin	mg dL^{-1}	0.2	15	7.6±4.6	7.8
Chol.	mg dL^{-1}	60	211	136±50	130
TG	mg dL^{-1}	29	456	136±126	88.5
LDL	mg dL^{-1}	26	122	62±27	38.5
HDL	mg dL^{-1}	20	46	35±9.8	58

*Duration of hemodialysis
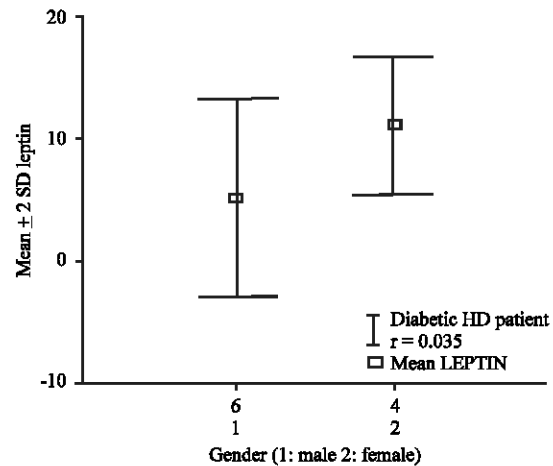
Fig. 1: Significant difference of serum leptin between males and females of diabetic HD population

non-diabetic groups were 7.63±4.63 (median: 7.7) and 6.85±10.47 (median: 3.45) ng mL^{-1}, respectively. The mean serum cholesterol levels of all the patients was 118±39 mg dL^{-1}. The mean serum cholesterol values with in the diabetic and non-diabetic groups were 136±50 and
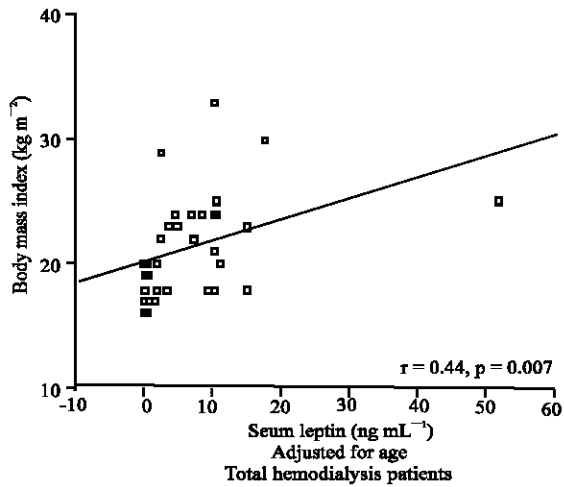
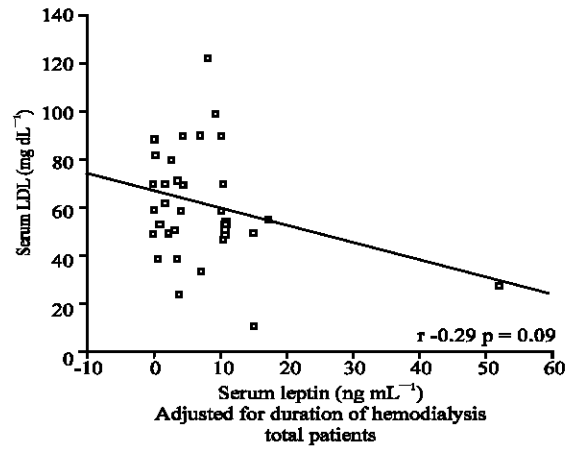Fig. 2: Significant positive correlation of serum leptin with BMI
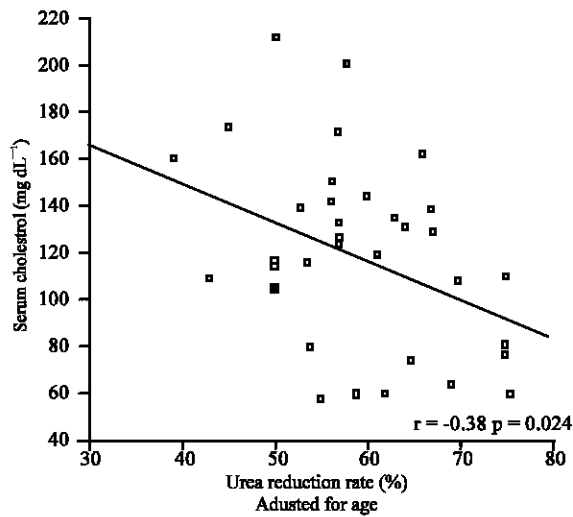

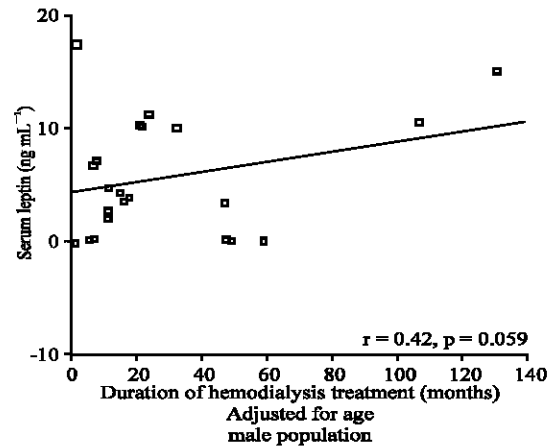Fig. 3: Significant inverse correlation of serum cholesterol with hemodialysis efficacy as determined by URR

Fig. 4: Near significant inverse correlation of serum leptin with serum LDL

Fig. 5: Significant positive correlation of serum leptin with duration of hemodialysis

Fig. 6: Significant positive correlation of serum leptin with age

111 ± 32 mg dL^{-1}, respectively. The mean BMI of all the patients was 21 ± 4 kg m^{-2}. The mean BMI with in the diabetic and non-diabetic groups were 22 ± 1.7 and 21 ± 4.5 kg m^{-2}, respectively. In this study, no significant differences between age, BMI, duration of hemodialysis treatment, dialysis dosage, URR, serum leptin, Tg, Chol, LDL-C, between males and female HD patients were seen non significant, However, there was a near significant difference of serum HDL-C between males and female HD patients (p = 0.058) were seen, also no significant between diabetic and non diabetic of female group was seen (p = 0.057). In this study no significant difference of serum leptin between males and females of
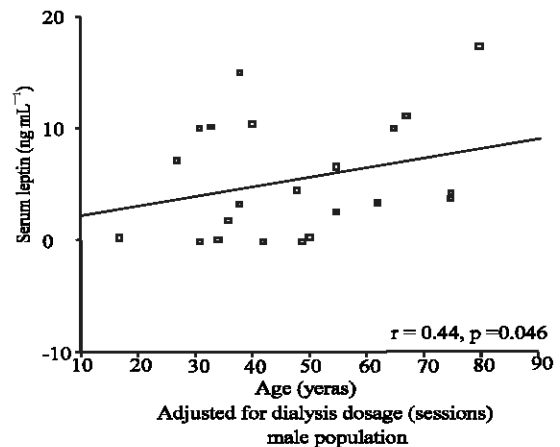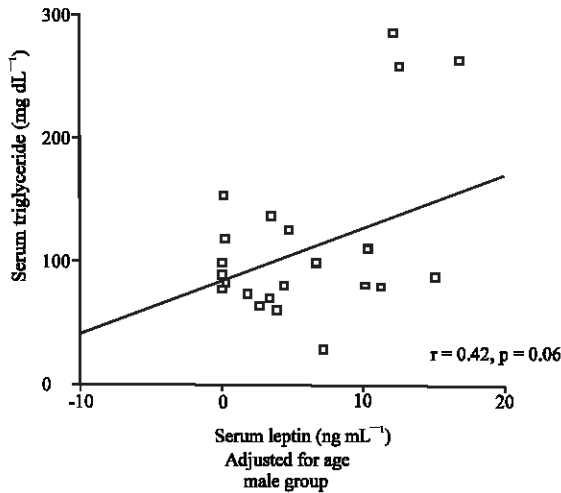
Fig. 7: Near significant positive correlation of serum leptin with serum triglyceride level

non diabetic HD patients was seen, however a significant difference of serum leptin between males and females of diabetic HD population was seen (r = 0.035; Fig. 1). In all patients a differences between age, BMI, DURATION of hemodialysis treatment, dialysis dosage, URR, serum leptin, TG, Chol, LDL-C and serum HDL-C between diabetic and non-diabetic HD patients were found non significant. There was a near significant difference of serum cholesterol significant positive correlation of serum leptin with BMI (r = 0.44, p = 0.007; Fig. 2) was seen. A significant positive correlation of serum cholesterol with serum triglyceride (r = 0.66, p<0.001) and a significant positive correlation of serum cholesterol with serum LDL-C (r = 0.58, p<0.001) and also a near significant positive correlation of serum LDL-C and BMI (r = 0.28, p = 0.093) were found. More over a near significant positive correlation of serum Chol with BMI (r = 0.28, p = 0.093) was seen too. A significant positive correlation of serum Tg with BMI (r = 0.54, p = 0.001) and a significant inverse correlations of serum Chol with duration (r = -0.36, p = 0.035) and dosage (r = -0.35, p = 0.035) of hemodialysis were found. A significant inverse correlation of serum Chol with hemodialysis efficacy as determined by URR (r = -0.38, p = 0.024; Fig. 3) was seen too(adjusted for age for all above correlations).In total patients also a near significant and inverse correlation of serum leptin with serum LDL-C (r = -0.29, p = 0.09; Fig. 4) (adjusted for duration of hemodialysis) and a near significant correlation of serum Tg with logarithm of leptin (r = 0.30, p = 0.078)were seen too(adjusted for age). In male hemodialysis group a significant positive correlation of serum leptin with duration of hemodialysis (r = 0.45,

p = 0.035; Fig. 5) (adjusted for age) and a significant positive correlation of serum leptin with ages of the patients (r = 0.44, p = 0.046; Fig. 6) (adjusted for dialysis dosage) were seen too, in this group also a near significant positive correlation of serum leptin with serum triglyceride levels (r = 0.42, p = 0.06; Fig. 7) (adjusted for age) was also seen. In total patients there was a near significant positive correlation of serum leptin with serum cholesterol of patients who had a cholesterol levels of more than 100 mg dL^{-1} (r = 0.35, p = 0.094) (adjusted for age).

DISCUSSION

In this study we found a significant positive correlation of serum leptin with BMI and a near significant positive correlations of serum LDL and Chol with BMI were seen too. Moreover a significant positive correlation of serum TG with BMI and a significant inverse correlations of serum Chol with duration and dosage of hemodialysis were seen too. In total patients also a near significant inverse correlation of serum leptin with serum LDL was seen. In male hemodialysis group a significant positive correlation of serum leptin with hemodialysis duration and also with the ages of the patients were seen. In this group also a near significant positive correlation of serum leptin with serum triglyceride level was seen. More over in total patients there was a near significant positive correlation of serum leptin with serum cholesterol of patients who had a cholesterol levels of more than 100 mg dL^{-1}. In a study conducted by Fox *et al.* on 812 incident hemodialysis found a 40% prevalence of hyperlipidemia in patients (Fox *et al.*, 2004). To compare 46 hemodialysis patients with 56 healthy subjects in the aspect of serum lipids, Gillett *et al.* (2004) showed that, total and LDL-cholesterol were unchanged, triacylglycerols and free cholesterol were raised and HDL-cholesterol concentrations were significantly decreased compared to controls. Previously we showed that on thirty-six patients with the mean of ages of 47.5 years old, the mean cholesterol level was 153.4±31.3 mg dL^{-1}, also the mean triglyceride level was 135.1±66.1 mg dL^{-1} (Nasri, and Baradaran, 2004). Concerning these results, studies showed that cholesterol levels is inversely associated with mortality in dialysis patients. This paradox may be explained by systemic inflammation and/or malnutrition, which are associated with lower cholesterol levels and higher mortality (Liu *et al.*, 2004). In fact dialysis patients have a high risk of atherosclerotic cardiovascular disease, but dialysis patients with higher serum cholesterol have lower mortality rates (Fox *et al.*, 2004). Recent

epidemiologic studies have suggested that increased serum levels of leptin may reduce nutrient intake and contribute to the development of protein-energy malnutrition (Mehrotra and Kopple, 2002) that may be shown by low levels of serum cholesterol. In contrast to our results that showed a near significant and inverse correlation of serum leptin with serum LDL and a near significant positive correlation of serum leptin with serum triglyceride and cholesterol levels, on 52 hemodialyzed individuals aged 24-74 years, Zbroch *et al.* (1999) found that serum lipid concentrations had no correlation with the serum concentrations of leptin. In another study conducted by Bossola *et al.* (2004) on 24 healthy subjects and in 49 end-stage renal disease patients on maintenance HD, no significant correlation between serum leptin level and cholesterol was found. In an agreement with our findings, Obineche *et al.* (2002) measured serum leptin levels in 150 patients on haemodialysis, peritoneal dialysis or in the predialysis phase of CRF, and observed significantly elevated levels of leptin, particularly in female patients and leptin was shown to correlate significantly with total and LDL-cholesterol. To compare serum levels of leptin, some nutritional parameters and serum lipids in hemodialysed patients (n = 46) and healthy subjects (n = 24) and to explore the relationship between serum leptin levels and the nutritional parameters in both groups, Svobodova *et al.* (2001) showed firstly the low serum triglyceride levels compared to controls and secondly serum leptin levels in hemodialysed patients correlated positively with serum cholesterol and triglyceride levels. Inverse correlations of serum cholesterol with duration and dosage of hemodialysis and with dialysis adequacy as well, may shows the effects of poorly adequate dialysis on aggravating the malnutrition syndrome and also dyslipidemia of hemodialysis as well. We showed the significant positive correlation of serum leptin with duration of hemodialysis that might potentiate the dyslipidemia in patients. Present data supports the hypothesis that in patients on hemodialysis, the association of leptin with cholesterol and triglyceride levels could show the positive effects of leptin on nutrition in hemodialysis patients which is in contrast to normal population.

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