

LEPTIN AND INTERLEUKIN-6 IN END-STAGE RENAL DISEASE

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ABSTRACT

Objective: Leptin, the product of ob gene, is important in control of appetite in humans. The objective was to determine the pre and post serum leptin levels in patients with ESRD (undergoing hemodialysis), to compare the pre serum leptin levels in patients with ESRD and normal healthy controls and to determine the serum IL-6 levels among patients with ESRD before and after hemodialysis.

Methodology: We measured the pre-and post dialysis leptin levels in hemodialysis patients (n=78) with end-stage renal disease (ESRD) with normal volunteers (n=78). Also plasma interleukin-6 was measured pre-and post dialysis in ESRD patients.

Results: Mean serum leptin levels were significantly higher in ESRD patients than in normal control subjects (38.22 ± 6.25 vs. 7.1 ± 4.38 ng/ml, respectively, $P < 0.01$). Serum leptin post-dialysis levels were significantly greater than pre-dialysis levels (44.78 ± 5.85 vs. 38.22 ± 6.25 ng/ml, respectively, $P < 0.05$). Post dialysis IL-6 levels was significantly greater than predialysis levels (14.7 ± 4.6 vs. 9 ± 4.9 pg/ml, respectively, $P < 0.01$).

Conclusion: Kidney contributes to clearance of circulating leptin in humans. However, further studies are needed to evaluate the significance of these elevated leptin levels in patients with end-stage renal disease.

KEYWORD: Leptin, Interleukin-6, ESRD Patients.

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INTRODUCTION

Leptin the 16-KDa product of obese gene, is currently believed to be involved in the regulation of appetite and energy expenditure.¹⁻³

Leptin is secreted exclusively by adipocytes^{4,5} and in normal humans; plasma concentrations are proportional to adipocytes. The physiological actions and the degradation pathways of leptin in humans are unknown.⁶ Several lines of indirect evidence suggest that renal clearance is the major route for leptin metabolism. Given the short half-life of leptin in the circulation and the presence of the leptin receptor in the kidney, it was postulated that the kidney serves as a site of clearance of leptin from the circulation.⁷ If leptin is cleared by the kidney we would expect to see a net uptake of leptin across the renal vascular bed and decreased renal uptake in patients with renal insufficiency. Furthermore, leptin levels would be expected to be elevated in chronic hemodi-

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alysis patients. Also elevated leptin levels may then have an important influence on the decreased appetite that is characteristic of patients with end-stage renal disease.

Indeed, in a recent longitudinal study it was found that increase serum leptin during peritoneal dialysis were associated with both inflammation and a loss of lean body mass.⁸ The purpose of this study was to determine the pre and post serum leptin levels in patients with ESRD (undergoing hemodialysis), to compare the pre serum leptin levels in patients with ESRD and normal healthy controls and to determine the serum IL-6 levels among patients with ESRD before and after hemodialysis. The importance of this study is that so far the above experiments have not been performed in the Iranian population.

METHODOLOGY

All subjects included in this study had given informed consent for participation and ethics committee approval was obtained. The study population consisted of 78 patients (48 males 61%, 30 females 38%; ages between 18-66) at Hemodialysis Unit, Tehran University Medical School between January 2006 and July 2007. Patients were excluded if they had been on dialysis for less than one year and patients on corticosteroid therapy due to a possible interaction of corticosteroids with leptin production.⁹ A separate 78 healthy subjects (45 male 57.69%, 33 females 42.30%; ages between 18-72) were enrolled in this study as normal controls.

Exclusion criteria was age more than 70 years and unwillingness to participate in the study. The dialysis filter used in all these patients was composed of modified cellulose membrane (Hemophane) with a pore size of 11,000 Daltons.

Aliquots taken from pre-and postdialysis plasma samples of ESRD patients were stored at -70°C and later used for leptin and IL-6 measurements. All specimens were collected during routine dialysis hours (0700-1600h), and the subjects received their regular diet and medications.

Plasma leptin levels were determined by commercially available highly sensitive ELISA kit (DRG International, Inc. USA).

The patient sample containing endogenous leptin was incubated with a specific rabbit anti leptin antibody. The sandwich complex is formed. The unbound material is washed off and an anti rabbit peroxidase conjugate is added for detection of the bound leptin which is proportional to the concentration of leptin in the patient sample.

Plasma IL-6 was measured by commercially available highly sensitivity ELISA (Bohringer Mannheim, Mannheim, Germany), and served as a marker of inflammation. This method applies a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with monoclonal antibody specific to IL-6. IL-6 if present, will bind and become immobilized by the antibody pre-coated on the wells and then be sandwiched by biotin conjugate. Avidin conjugate to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The wells are thoroughly washed and only wells that contain IL-6, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour which is measured spectrophotometrically at a wavelength of 450nm.

Statistical Analysis: Statistical analysis were performed using SPSS software version 12.0. All results are given as mean \pm SD. Data were analysed using "paired analysis" to measure and compare the pre and post serum levels of leptin and interleukin-6 in ESRD patients undergoing hemodialysis.

RESULTS

Leptin levels were evaluated pre-and post-dialysis to evaluate if leptin may be cleared during hemodialysis. The dialysis membranes used in all patients was a modified cellulose membrane (Hemophane) with a pore size of 11,000 Dalton. As would be predicted by the molecular weight of leptin (14 to 16,000 Daltons), there was no evidence of clearance across the dialysis membrane with the post-dialysis levels significantly greater than pre-dialysis levels

(44.78± 5.85 vs. 38.22±6.25 ng/ml, respectively P<0.05).

Predialysis plasma leptin concentrations in ESRD patients were significantly higher than those obtained in normal volunteers (38.22±6.25 vs. 7.1±4.38 ng/ml, respectively, P<0.01). Post-dialysis IL-6 levels was significantly greater than predialysis levels (14.7± 4.6 vs. 9±4.9 pg/ml, respectively P<0.01). There is a positive correlation between leptin and IL-6 (P<0.05) in ESRD patients.

DISCUSSION

Leptin is primarily cleared by the kidney, therefore leptin levels are increased in patients with chronic renal disease or ESRD.^{7,10,11} Serum leptin levels correlate negatively with glomerular filtration rate (GFR) in patients with varying degrees of chronic renal insufficiency. Leptin accumulates in patients with ESRD compared to healthy controls.^{7,12,13} Multiple studies show that a decrease in GFR leads to the accumulation of leptin.^{7,12}

In dialysis patients high-flux membranes lower leptin levels by approximately 30% whereas low-flux membranes have not been shown to eliminate the molecule.^{7,13,14} Elevated leptin levels are seen in patients undergoing maintenance peritoneal dialysis as measured peritoneal clearances of 0.7-1.7ml/min have been noted.^{15,16} Given that the kidney is one of several peripheral tissues that exhibit the leptin receptor and the size of leptin allows it to cross the glomerular capillary basement membrane, we hypothesized that the kidney may be a major organ accounting for removal of leptin from the circulation. Our study demonstrates that there is net renal extraction of leptin in patients with normal renal function, where as this renal extraction is impaired in patients with mild to moderate renal insufficiency. Circulating leptin is not removed by the modified cellulose dialysis membrane used in our patients, presumably due to pore size of the membrane. Our findings are in agreement with a study by sharma et al.,⁷ which showed that plasma leptin is partly cleared by the kidney and is elevated in hemodialysis patients. Several

studies have shown that patients receiving maintenance hemodialysis and peritoneal dialysis have elevated levels of inflammatory mediators.^{11,17,18}

The pro-inflammatory cytokines are known to reduce feeding behavior in animals and along with leptin have been implicated in the development of malnutrition in ESRD patients.¹⁰ In the present study a significant positive correlation was observed between IL-6 and serum leptin levels, suggesting that inflammation may contribute to hyper leptinemia or vice versa in ESRD patients. The hypothesis that inflammation stimulates leptin production is not original, and several lines of evidence in the literature suggest that an acute phase response may stimulate leptin production.

Elevated leptin mRNA levels was observed in ESRD patients with an inflammatory response¹⁹ and Fouque et al.,²⁰ presented preliminary data showing a positive correlation between IL-6 and serum leptin in a group of hemodialysis patients. In several animal studies it has been demonstrated that cytokines raise leptin mRNA levels.²¹⁻²³ Moreover in humans, the administration of pro-inflammatory cytokines has been shown to increase serum leptin levels^{24,25} and in patients with sepsis leptin levels are elevated.²³ Finally, as leptin and its receptors share structural and functional similarities with members of the cytokine family, a cross-reaction between IL-6 and leptin may occur.

Furthermore IL-6 belongs to a family of 20KDa polypeptide cytokines, is a marker of inflammation in ESRD patients. Although the precise mechanisms, that are responsible for inflammation in ESRD patients is unclear, low grade infection, repeated exposure to dialysis filters, and auto oxidation products are considered as likely inciting factors in these patients.²⁶ Inflammation plays a primary role in arterial damage in dialysis patients. It is therefore of interest that elevated concentrations of serum IL-6 is a strong independent predictor of all-cause mortality in dialysis patients. More over it has been recently shown that elevated traditional risk factors, predict accelerated

atherosclerosis in patients on maintenance dialysis.²⁷

In conclusion the kidney contributes to clearance of circulating leptin in humans and further studies are required to evaluate the significance of these elevated leptin levels in relation to indices of appetite in patients with end-stage renal disease. Future research should also focus on elucidating the etiology of the inflammation and studying the long-term effects of various anti-inflammatory treatment strategies on nutritional and cardiovascular status as well as outcome in ESRD patients.

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