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Positive Correlation of Serum Leptin with Blood Lymphocytes in Maintenance Hemodialysis Patients

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KEY WORDS: leptin, lymphocyteds, hemodialysis

ABSTRACT

Objective: The objective of this study was to find the association of serum leptin with blood lymphocyte and polymorphonuclear (PMN) percentages as the markers of immune system function as well as nutritional status in maintenance hemodialysis patients.

Design: In a group of maintenance hemodialysis patients, serum leptin, albumin, creatinine, blood urea nitrogen, and white blood cell counts consisting of lymphocytes and PMN cells were measured.

Results: A significant positive correlation of serum leptin with body mass index, a significant positive correlation of serum leptin with lymphocyte percentage, and a significant inverse correlation of serum leptin with PMN percentage were seen. Near significant inverse correlations of white blood cell counts with duration and dosage of dialysis, a near significant inverse correlation of white blood cell counts with hemodialysis adequacy, and a significant inverse correlation of white blood cell counts with serum albumin were seen.

Conclusion: Generally, increased neutrophil counts and reduced lymphocyte counts are independent predictors of increased mortality risk in hemodialysis patients. Although this study and others showed positive association of serum leptin with lymphocytes and an inverse correlation of serum leptin with PMNs, the authors conclude a protective role for leptin in decreasing mortality in hemodialysis patients; therefore, serum leptin in hemodialysis has a reverse epidemiology role for maintaining immune system function in hemodialysis.

INTRODUCTION

Patients on chronic hemodialysis suffer from general immune incompetence.[1] Malnutrition as a cause of immune incompetence in dialysis patients is a common clinical problem in patients with end-stage renal disease (ESRD) and is generally the result of poor food intake.[2-3] Malnutrition is an independent factor causing morbidity and mortality.[4] Leptin is an adipocyte-secreted hormone that centrally regulates weight control.[5] However, leptin receptor is

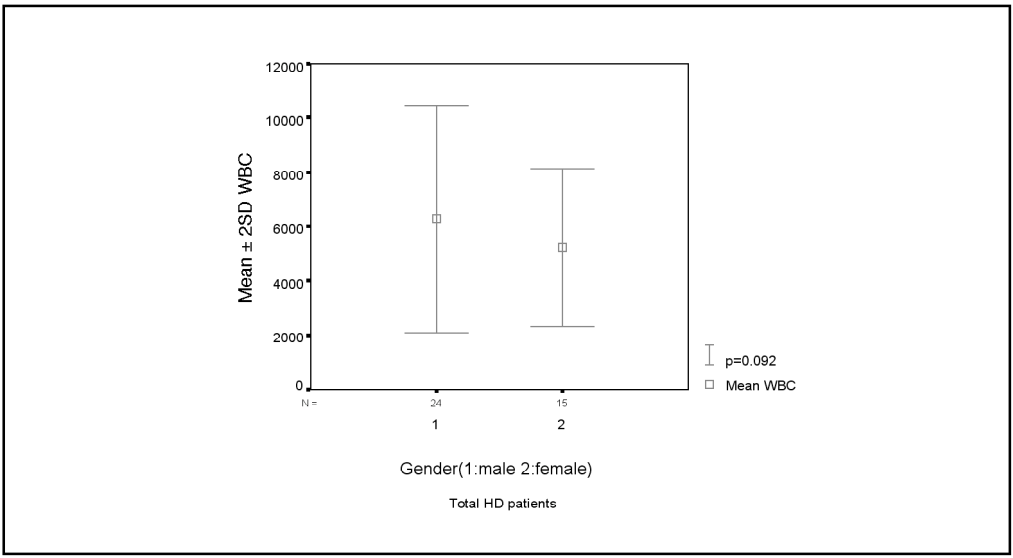

Figure 1: Near significant difference of white blood cell (WBC) counts between males and female hemodialysis (HD) patients.

expressed not only in the central nervous system, but also in other systems such as hematopoietic tissues.

Human leptin has previously been shown to enhance cytokine production by murine peritoneal macrophages and human circulating monocytes.[6] Leptin belongs to the helical cytokine family and its plasma concentrations correlate with fat mass and respond to changes in energy balance. Initially, leptin was considered an anti-obesity hormone, but experimental evidence has shown pleiotropic effects of this molecule on hematopoiesis, angiogenesis, lymphoid organ homeostasis, and T-lymphocyte functions. More specifically, leptin links the pro-inflammatory T-helper (Th)-1 immune response to nutritional status and energy balance. Indeed, decreased leptin concentrations during conditions of food deprivation lead to impaired immune capabilities.[7] Malnutrition and the consequent reduction of the fat mass cause immunodeficiency in animals and humans.[8-9] Reports have recently shown that leptin deficiency is responsible for the immunosuppression and the thymic atrophy observed during acute starvation and undernutrition.[10-11]

After nutritional deprivation, leptin blood levels fall because of reductions in body fat, causing impairment of the immune function. This effect also has been demonstrated in animals distant in the evolutionary scale such as insects.[12] Therefore, leptin seems to be one of the major players in the immunoendocrine scenario, regulating the correlation among nutritional status, basal metabolism, and immune function.[10]

Furthermore, the presence of leptin is necessary for an effective cell-mediated immune response.[10] Indeed, $CD4^+$ T-lymphocyte activities are suboptimal in the absence of leptin.[10] Serum leptin levels are elevated in patients with ESRD and in hemodialysis, and experimental evidence suggests a possible role for leptin in the development of protein-energy malnutrition in this population.[13-14] Indeed, in the last few years, we have developed an increasing understanding of how immunosuppression occurs during malnutrition.

The focus has shifted from nutrients to hormones, in particular leptin. Leptin induces oxidative stress in human endothelial cells. It is possible that high leptin levels in renal failure may further

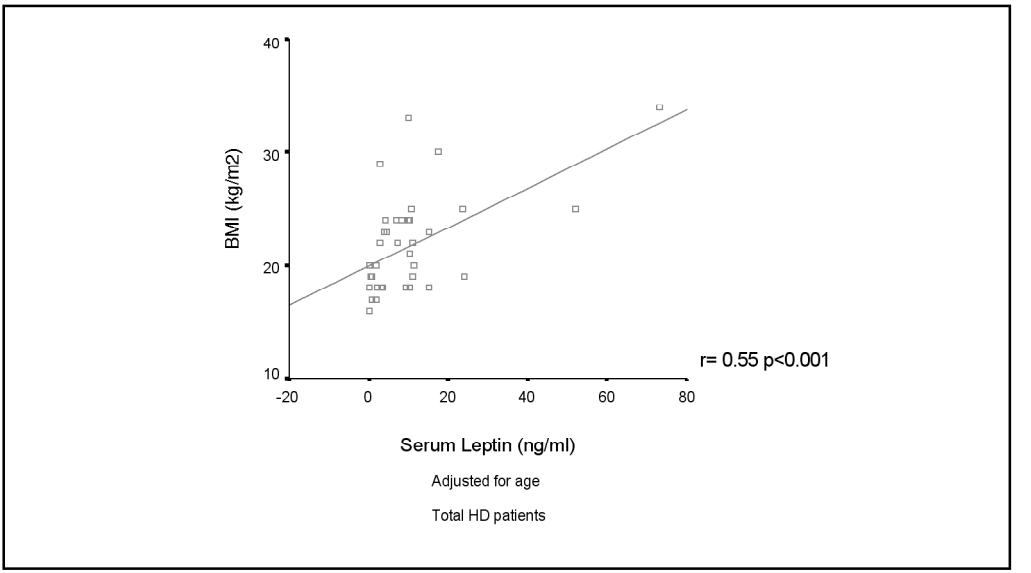

Figure 2: Significant positive correlation of serum leptin and body mass index

enhance renal oxidative stress.[15] Release of leptin from adipocytes may be stimulated by cytokines mediating the inflammatory response, which is frequently pronounced in patients with ESRD receiving hemodialysis.[14-16] Oxidative stress and an increased total white blood cell (WBC) count has been found to correlate with increased cardiovascular mortality in elderly men.[17] An association between WBC counts and mortality in ESRD also has been suggested.[17] To find the association of serum leptin with blood lymphocyte and PMN percentage as the markers of immune system function as well as nutritional status and while a high WBC and a low percentage of lymphocytes are associated with a significant increase in mortality and hospitalization in maintenance hemodialysis patients,[18] we therefore aimed to conduct a study on ESRD patients under regular hemodialysis to explore this association.

PATIENTS AND METHODS

This cross-sectional study was conducted on patients with ESRD who were undergoing maintenance hemodialysis treatment with acetate-based dialysate and polysulfone membranes. According to the severity of secondary hyperparathyroidism, each patient being treated for secondary hyperparathyroidism was given oral active vitamin D3 (Calcitriol; Rocaltrol) (Roche Hexagon; Roche Laboratories Inc , New Jersey), calcium carbonate capsule, and Rena-Gel (sevelamer; Genzyme Europe B.V.; United Kingdom/Ireland) tablet at various doses. According to the severity of anemia, patients were prescribed intravenous iron therapy with Iron Sucrose (Venofer; Vifor (international) Inc. St. Gallen, Switzerland) at various doses after each dialysis session. All patients received treatments of 6 mg folic acid daily, 500 mg Acetyl- L-Carnitine(Jarrow Formulas, Inc, Los Angeles, CA**)** daily, oral vitamin B-complex tablets daily, and 2,000 U intravenous Eprex (recombinant human erythropoietin [Rhuepo] Janssen-Cilag; CILAG- AG International 6300 Zug, Switzerland) after each dialysis session.

Exclusion criteria were active or chronic infection and using drugs had adverse effects on bone marrow.

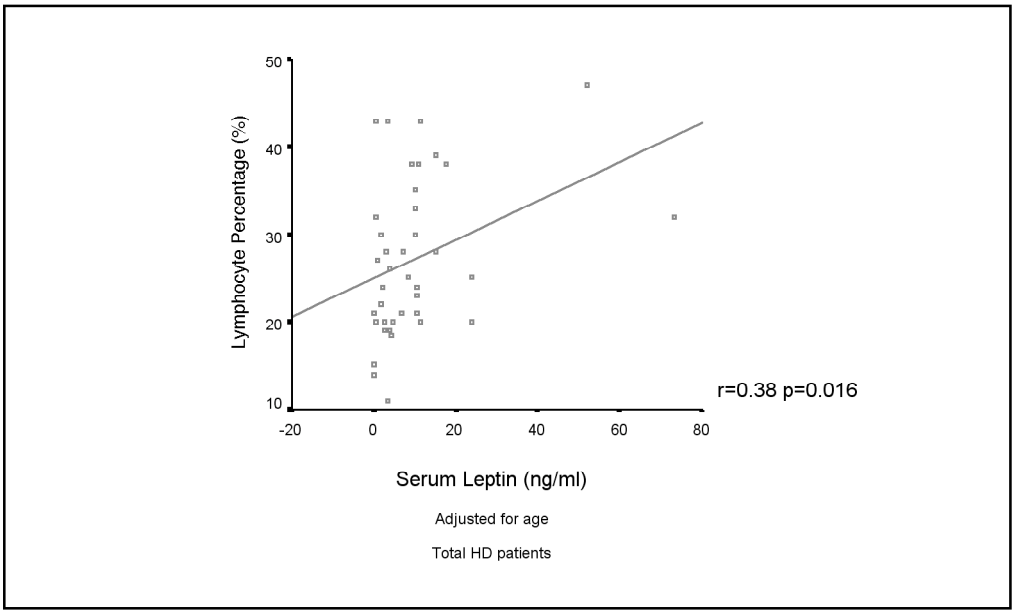

Figure 3: Significant positive correlation of serum leptin with lymphocyte percentage.

Complete blood counts containing white blood cells (WBCs/uL) with lymphocyte and PMN cells differentiation were measured using Sysmex-KX-21N cell counter **(**SYSMEX CORPORATION; Mundelein, Illinois, Sysmex America, Inc.**)**. Levels of serum predialysis creatinine and post- and predialysis BUN were measured using standard kits. Serum leptin (normal range of values for men is 3.84 ng/mL [±1.79] and for women is 7.36 ng/mL [±3.73]) was measured by enzyme-linked immunosorbent assay (ELISA) using DRG kits **(**DRG Diagnostics, Berlin, Germany**)**. The body mass index (BMI) was calculated using the standard formula (postdialyzed weight in kg/m^2).[19] For the efficacy of hemodialysis, the urea reduction rate (URR) was calculated from pre- and post-BUN data.[20]

Duration and dosages of hemodialysis treatment were calculated from the patients' records. The duration of each hemodialysis session was 4 hours. Statistical analysis was performed on total hemodialysis (HD), female, male, diabetic, and nondiabetic populations separately. For statistical analysis, the data are expressed as the mean ± standard deviation (SD). Comparison between the groups was done using Student's *t*-test. Statistical correlations were assessed using partial correlation test. All statistical analyses were performed using SPSS software (version 11.5.00) (SPSS Inc,Chicago,IL). Statistical significance was determined at a P value <0.05.

RESULTS

There was a total of 39 patients (15 women, 24 men), consisting of 27 nondiabetic HD patients (11 women, 16 men) and 12 diabetic HD patients (4 women, 8 men). Table 1 shows the patients' mean ± SD age, the length of time they were on hemodialysis, the dialysis dosage, and the results of laboratory tests. The mean patient age was 46 years (±18).

The value of serum leptin of total patients was 10 ng/mL (±14) (median 6.8 ng/mL). The percentage of lymphocytes

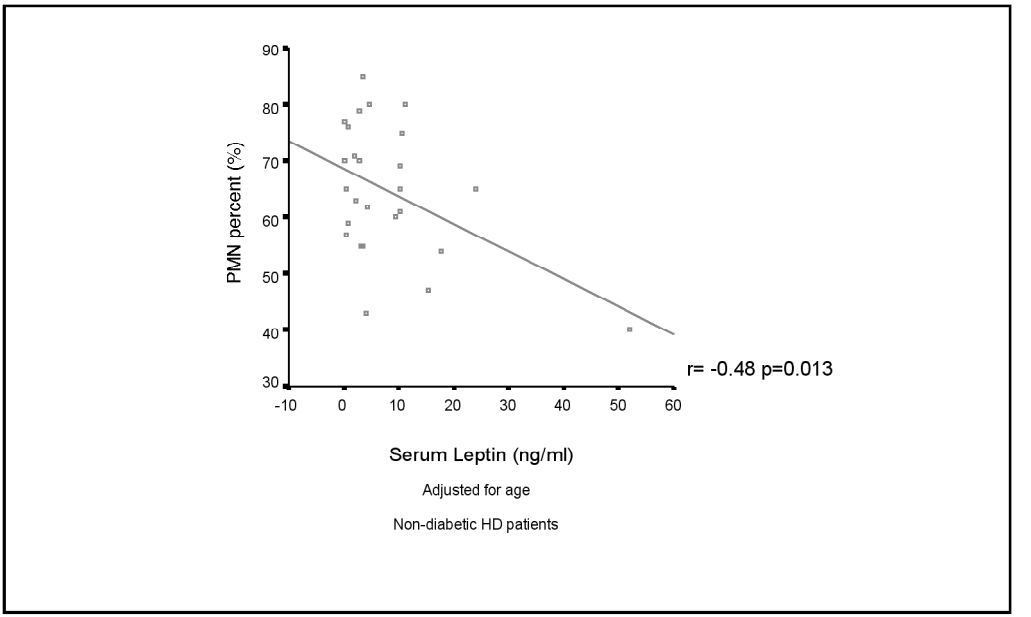

Figure 4: Significant inverse correlation of serum leptin with PMN percentage.

in total patients was 27% (±9) (median 25%). In all patients no significant differences of lymphocyte and PMN percentage and serum leptin between men and women were found. However, a near significant difference of WBC counts between men and women was seen (*P*=0.092; Figure 1). No significant differences of WBC counts, lymphocyte, PMN percentage, and serum leptin between diabetic and nondiabetic HD patients were seen.

A significant positive correlation of serum leptin with BMI (*r*=0.55; *P*<0.001; Figure 2) was seen. Although in total patients there was a significant positive correlation of serum leptin with lymphocyte percentage (*r*=0.38; *P*=0.016; Figure 3), only in nondiabetic HD patients were found a significant inverse correlation of serum leptin with PMN percentage (*r*= -0.48; *P*=0.013; Figure 4). In all patients, no significant correlations of WBC counts, lymphocyte, and PMN percentage with BMI were seen. In all patients, a near significant and inverse correlation of WBC counts with duration of hemodialysis (*r*=-0.27; *P*=0.094) and in female population a near significant and inverse correlation of WBC counts with hemodialysis dosage were seen (*r*=-0.52; *P*=0.055).

In all patients a near significant and inverse correlation of WBC counts with hemodialysis adequacy as determined by URR (*r*=-0.29; *P*=0.072) was seen (adjusted for age for all correlations). The association of dialysis adequacy (by URR) with WBC counts in the male HD group was positively significant (*r*=0.49; *P*=0.017; Figure 5) (adjusted for dialysis dosage).

DISCUSSION

In this study we found a near significant difference of WBC counts between male and female dialysis patients with more values among men. There was a significant positive correlation of serum leptin with BMI, a significant positive correlation of serum leptin with lymphocyte percentage, and a significant inverse correlation of serum leptin with PMN percentage. We also found a near significant

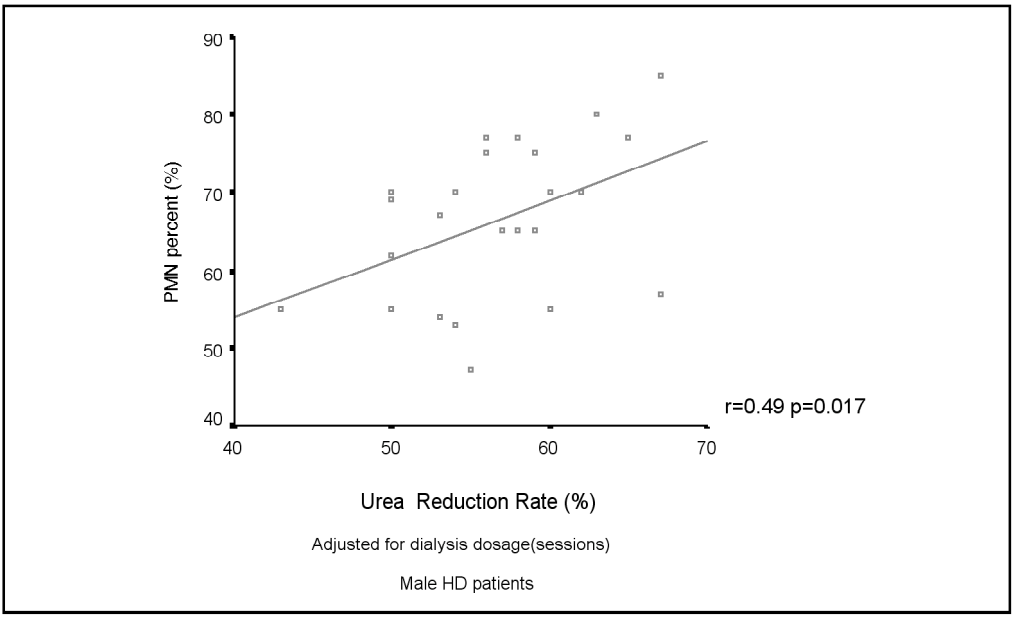

Figure 5: Significant positive correlation of dialysis adequacy by urea reduction rate (URR) with WBC counts .

inverse correlation of WBC counts with duration of hemodialysis, a near significant and inverse correlation of WBC counts with hemodialysis dosage, and a near significant and inverse correlation of WBC counts with hemodialysis adequacy.

Leptin was found to be in direct proportion to the amount and percentage of body fat.[21] Leptin and the leptin receptor are part of a novel pathway that stimulates haemopoiesis.[21] Leptin signals through leptin receptor (Ob-R), which is a member of a class I cytokine receptor family.[22] It has been reported that Ob-R is expressed also in human CD34+ cells and that leptin administration induces proliferation of human and murine stem-cells in vitro.[23] Consistent with our finding in HD patients, Mabuchi and colleagues, endeavoring to find the relation of WBC count and serum leptin concentrations, conducted a study with 1,082 men and 200 women aged 40 to 59 years (normal subjects) and showed a significant and independent association of WBC count and serum leptin concentration.[24] In this regard, in a population of 44,114 ESRD patients receiving hemodialysis, Reddan and colleagues[25] found the following:

- higher lymphocyte count was associated with higher serum albumin and creatinine,
- high neutrophil count was associated with lower serum albumin and creatinine,
- increased lymphocyte count was associated with reduced mortality risk, and
- increased neutrophil count was associated with increased mortality risk.

In addition, it was shown that an increased neutrophil counts is strongly associated with, and reduced lymphocyte counts associated less strongly with, many surrogates of both malnutrition and inflammation. An increased neutrophil count and reduced lymphocyte count are independent predictors of increased mortality risk in hemodialysis patients.[25]

In our study, we also demonstrated the negative association of leptin with

Table 1. Minimum and maximum of age, duration and dosage hemodialysis, and laboratory results of hemodialysis patients

Total patients				
N=39	**Minimum**	**Maximum**	**Mean±SD**	**Median**
Age years	16	80	46±18	42
DH* months	2	156	31±35	18
Dialysis				
dose (sessions)	36	1584	279±381	156
URR %	39	76	58.7±8.7	58
BMI kg/m^2	16	34	21.7±4.4	21
Leptin ng/mL	0.10	73	10±14	6.8
Creat mg/dL	3	18	9.5±3.6	9
BUN mg/dL	30	180	83±33	76
WBC counts/ uL	3000	11200	4860±2710	5500
Lymphocyte %	11	47	27.2±8.9	25
PMN %	40	85	65.3±11	67
Non-diabetic HD patients				
n=27	**Minimum**	**Maximum**	**Mean±SD**	**Median**
Age years	16	80	42±17	49
DH* months	2	156	37±40	21
Dialysis				
dose (sessions)	36	1584	347±442	156
URR %	50	76	60±7.6	60
BMI kg/m^2	16	33	21±4.4	19
Leptin ng/mL	0.10	52	8±10	4
Creat mg/dL	4	15	9.8±3	10
BUN mg/dL	30	180	82±32	74
WBC counts/ uL	3000	10300	5600±1800	5500
Lymphocyte %	11	47	27±9	25
PMN %	40	85	64.8±11.5	65
Diabetic HD patients				
n=12	**Minimum**	**Maximum**	**Mean±SD**	**Median**
Age years	27	79	55±17	57
DH* months	6	24	15±6	15
Dialysis				
dose (sessions)	54	216	127±59	132
URR %	39	75	54±9.5	54
BMI kg/m^2	20	34	23±3.7	23
Leptin ng/mL	0.20	73	14.4±19.5	9.4
Creat mg/dL	3	17	8.7±4.7	9
BUN mg/dL	30	140	85±35	90
WBC counts /uL	3500	11200	4860±2710	6200
Lymphocyte %	15	43	51±18	26.5
PMN %	42	77	66.5±10.8	70

*Duration of hemodialysis treatment

PMN percentage. Negative associations of WBC counts with duration and doses of dialysis are the result of further toxic and suppressive effects of uremic toxins on bone marrow with increasing the duration of dialysis. Also, a positive correlation of WBC counts with dialysis efficacy may show that an adequate dialysis acts toward the resorting of a hematopoetic system.

As was explained regarding the association of nutritional deprivation and leptin levels in subjects without renal failure, leptin blood levels fall because of a reduction in body fat, causing impairment of the immune function.[8-12] It also has been described in humans that leptin deficiency causes increased frequency of and mortality by infections early in life.[26] In conditions of nutritional deficit and reduced energy stores, leptin plays a crucial function that contributes to induce all the adaptive mechanisms necessary to save energy, ensuring the correct function of vital organs such as the heart, kidney, and brain.[27] Indeed, the organism through leptin deficiency reduces activation and expansion of immune cells to sustain more life-necessary functions.

Conversely, in conditions of excess and unbalanced leptin signals, together with genetic, gender, and environmental factors, leptin can favor the break of self-tolerance and at least in some animal models can sustain and promote $CD4^+$ T-cell-mediated autoimmune diseases.[28] To answer whether leptin plays a role in the immunosuppression of malnutrition in humans, Palacio and colleagues studied children with protein-calorie malnutrition, who had slightly decreased fat and significantly lower leptin levels.[29] This study showed that there was no correlation between body fat and leptin, reflecting the acute suppressive effects of malnutrition. With feeding, leptin levels rapidly returned to normal, with restoration of the correlation to body fat before restoration of normal fat content. Likewise with feeding, the ability of the infants' polymorphonuclear white cells to make interferon-γ and TNF-α increased, whereas production of IL-4 decreased. These changes are similar, but not identical with what is found with leptin treatment of *ob/ob (obese* (*ob*) mouse strain) and starved mice.[29]

High leptin levels signal the presence of sufficient energy stores to sites in the central nervous system, which respond by reducing appetite and increasing energy expenditure, preventing severe obesity.[30] Therefore, leptin signals the nutritional status from the periphery to the area of the brain involved in the homeostasis of energy balance.[30]

The increased levels of leptin in hemodialysis patients are not only the result of retention of the hormone, but probably from increased production. Anorexia of hemodialysis patients has been attributed to the increased leptin levels, even if this is largely a hypothesis.[31] We saw that in subjects without renal failure, malnutrition was associated with leptin deficiency. In contrast to normal subjects, in hemodialysis patients malnutrition was associated with hyperleptinemia, which may be the result of microinflamation status caused by hemodialysis treatment and the inflammatory stimuli, which have previously been shown to induce elevated systemic leptin concentrations, proposing that leptin induction is part of the ubiquitous acute phase reaction. This has been explained by the cytokine properties of leptin and its receptor, as the secondary structure of leptin resembles that of cytokines and the leptin receptor is homologous to the signal-transducing subunit of the IL-6 receptor family.[32-34]

We showed a significant positive correlation of serum leptin with BMI.

More recent studies in maintenance dialysis patients suggest a paradoxically inverse association between higher serum leptin and improved markers of nutritional status.[33-34] This finding is consistent with the theory of reverse epidemiology for leptin.[33-34] Indeed, leptin has been reported to be a negative acute phase reactant in ESRD patients.[30] Thus, while an increased neutrophil count and reduced lymphocyte count are independent predictors of increased mortality risk in hemodialysis patients, and while our study and others showed positive association of serum leptin with lymphocytes, and inverse correlation of serum leptin with PMNs, we can conclude a protective role for leptin in decreasing mortality in hemodialysis patients and therefore serum leptin in hemodialysis has a reverse epidemiology role for maintaining the immune system in hemodialysis.

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