CLINICAL STUDY

The Association between Serum Leptin and Blood Lymphocytes in Hemodialysis Patients

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Abstract

Aims: To find the association between serum leptin, blood lymphocytes and PMN percentages as markers of immune-system function, as well as nutritional status in long term hemodialysis patients.

Patients and methods: In a group of long term hemodialysis patients, serum leptin, albumin, creatinine, BUN, and white-blood cell (WBC) count - [lymphocytes and polymorphonuclear (PMN) cells] were measured.

Results: A significant positive correlation between serum leptin and body-mass index and between serum leptin and lymphocyte percentage was found, as well as a significant negative correlation between serum leptin and PMN percentage. There was a weak negative correlation between WBC counts and the duration and dosage of dialysis, and also a near significant negative correlation between WBC counts and hemodialysis adequacy. There was also a significant negative correlation between WBC counts and serum albumin.

Conclusion: Generally increased neutrophil count and increased lymphocyte count markers of an increased mortality in hemodialysis patients. This study shows a positive association between serum leptin and lymphocytes, and a negative correlation between serum leptin and PMN. Leptin might have a protective role in decreasing mortality in hemodialysis patients by maintaining the function of the immune system (Tab. 1, Fig. 3, Ref. 33). Full Text (Free, PDF) www.bmj.sk.

Key words: end-stage renal failure, hemodialysis, serum leptin, lymphocyte percentage, white-blood cell count, reverse epidemiology.

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 due to 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 expressed not only in the central nervous system, but also in other systems such as hematopoetic 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 as an anti-obesity hormone, but experimental evidence has also shown pleiotropic effects of this molecule on hematopoiesis, angiogenesis, lymphoid organ homeostasis and T lymphocyte functions as mentioned above. More specifically, leptin links the pro-inflammatory T helper (Th) 1 immune response to the nutritional status and the energy balance. Indeed, decreased leptin concentrations during conditions of food deprivation lead to impaired immune capabilities (7).

Malnutrition and consequent reduction of the fat mass causes 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 malnutrition (10, 11). Following malnutrition,
Tab. 1. Mean±SD, Minimum and Maximum of age, duration and dosage of hemodialysis and also laboratory results of hemodialysis patients.

<table>
<thead>
<tr>
<th></th>
<th>Total patients</th>
<th>Non-Diabetic HD patients</th>
<th>Diabetic HD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=39</td>
<td>n=27</td>
<td>n=12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16±80</td>
<td>16±80</td>
<td>27±79</td>
</tr>
<tr>
<td>DH* (months)</td>
<td>2±156</td>
<td>2±156</td>
<td>6±24</td>
</tr>
<tr>
<td>Dialysis dose sessions</td>
<td>36±1584</td>
<td>36±1584</td>
<td>54±216</td>
</tr>
<tr>
<td>URR (%)</td>
<td>39±76</td>
<td>50±76</td>
<td>39±75</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16±34</td>
<td>16±33</td>
<td>20±34</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>0.10±73</td>
<td>0.10±52</td>
<td>0.20±73</td>
</tr>
<tr>
<td>Creat (mg/dl)</td>
<td>3±18</td>
<td>4±15</td>
<td>3±17</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>30±180</td>
<td>30±180</td>
<td>30±140</td>
</tr>
<tr>
<td>WBC (counts/µl)</td>
<td>3000±11200</td>
<td>3000±10300</td>
<td>3500±11200</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>11±47</td>
<td>11±47</td>
<td>15±43</td>
</tr>
<tr>
<td>PMN (%)</td>
<td>40±85</td>
<td>40±85</td>
<td>42±77</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>46±18</td>
<td>347±442</td>
<td>55±17</td>
</tr>
<tr>
<td>Median</td>
<td>42</td>
<td>156</td>
<td>57</td>
</tr>
</tbody>
</table>

* – duration of hemodialysis

leptin serum level falls due to reduction in body fat causing impairment of the immune function. This effect has also 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 end-stage renal disease (ESRD) and in hemodialysis. Experimental evidence suggests a possible role of leptin in the development of protein-energy malnutrition in this population (13, 14). Indeed, in the last few years, we have understood how immunosuppression develops during malnutrition. The focus has been shifted from nutrients to hormones, in particular to leptin. Leptin induces oxidative stress in human endothelial cells. It is possible that high leptin levels in renal failure may further 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 end-stage renal disease on hemodialysis (14–16). Oxidative stress and an increased total white blood cell (WBC) count has been found to correlate with an increased cardiovascular mortality in elderly men (17). An association between WBC count and mortality in ESRD has also been suggested (17).
This study assessed the association between serum leptin, blood lymphocytes and PMN percentage as the markers of immune-system function, as well as nutritional status. Because elevated WBC count and a low percentage of lymphocytes are associated with a significant increase in mortality and hospitalization in long term hemodialysis patients (18), we conducted a study on ESRD patients undergoing regular hemodialysis to assess this association.

Patients and methods

This cross-sectional study was conducted on patients with end-stage renal disease (ESRD), who were undergoing long term hemodialysis (HD) treatment with acetate basis dialysate and polysulfone membranes. According to the severity of secondary hyperparathyroidism, those patients who were being treated for secondary hyperparathyroidism were given oral active vitamin D3 (Rocaltril), calcium carbonate, and Rena-Gel capsules at various doses. According to the severity of anemia, patients were undergoing i.v. iron therapy with iron sucrose (venofer) at various doses after each dialysis session. In addition, all patients under treatment, received 6 mg/day of folic acid, 500 mg L-carnitine per day, an oral vitamin B-complex tablet daily, and 2000 U (i.v.) of Eprex [recombinant human erythropoietin (rHuEPO)] after each dialysis session. Exclusion criteria were active or chronic infection and the use of drugs that had adverse effects on bone marrow. White blood counts were made (WBCs/µl), and lymphocyte and polymorphonuclear cell (PMNs) differentiation was measured using a Sysmex-KX-21N Cell counter. Levels of serum pre-dialysis creatinine (Creat), and post- and pre-dialysis blood urea nitrogen (BUN) were measured using standard kits.

Serum leptin [normal range of 3.84 (±1.79) for males and 7.36 (±3.73) ng/ml for females] was measured by an enzyme-linked immunosorbent assay (ELISA), using DRG kits from Germany. The body-mass index (BMI) was calculated using a standard formula (post-dialyzed weight in kilograms/height in meters to give the mass per m²) (19). In regards to the efficacy of hemodialysis, the urea-reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (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 analyses were performed on all hemodialysis (HD) patients, as well as females, males, diabetics and non-diabetics separately.

For statistical analysis, the data 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 a partial correlation test. All statistical analyses were performed using SPSS (version 11.5.00). Statistical significance was determined at a p-value <0.05.

Results

There were a total of 39 patients [15 females (F), 24 males (M)], consisting of 27 (11 F, 16 M) non-diabetic HD patients and 12 (4 F, 8 M) diabetic HD patients. Table 1 shows the patients’ mean age, the length of time they were on hemodialysis, their previous dialysis sessions, and the results of the laboratory tests.

The patient’s mean age was 46 (±18) years.

The value of serum leptin for all patients was 10 (±14) ng/ml (median: 6.8 ng/ml). The mean percentage of lymphocytes in all patients was 27 (±9) % (median 25 %). There were no significant differences between lymphocyte, PMN percentage and serum leptin between males and females. No significant differences in WBC counts, lymphocytes, PMN percentage and serum leptin between diabetic and non-diabetic HD patients were found (p N.S.).

A significant positive correlation of logarithm of serum leptin and body-mass index (r=0.55, p<0.001) (Fig. 1) (adjusted for age) was seen in all patients. In all patients, also a significant positive
Leptin and the leptin receptor are the part of a novel pathway which 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 (21). Consistent with our finding in HD patients, Mabuchi et al tried to find the relation of WBC count and serum leptin concentrations. He has conducted a study on 1082 men and 200 women aged 40 to 59 years (normal subjects) and showed significant and independent association of WBC count and serum leptin concentration (23). Studies shown that white blood cell (WBC) counts is correlated to the amount of body fat in humans. In this regard, in the population of 44114 ESRD patients receiving haemodialysis, Reddan et al found that a higher lymphocyte count was associated with higher serum albumin and creatinine and a high neutrophil count was associated with lower serum albumin and creatinine, and also an increased lymphocyte counts was associated with reduced mortality risk and an increased neutrophil counts was associated with increased mortality risk. It was shown that an increased neutrophil counts is strongly associated and reduced lymphocyte counts less strongly associated with many surrogates of both malnutrition and inflammation. An increased neutrophil counts and reduced lymphocyte counts are independent predictors of reduced mortality risk in haemodialysis patients (24). In our study, we also showed the negative association between Leptin and PMN percentage. In our study, the negative associations between WBC counts and duration and doses of dialysis is caused by further toxic and suppressive effects of uremic toxins on bone marrow increasing with the duration of dialysis. Also, the positive correlation of WBC counts to dialysis efficacy may show that an adequate dialysis acts toward the resorting of hematopoietic system. As explained in the association of nutritional deprivations and leptin levels in subjects without renal failure, serum levels fall due to reduction in body fat, causing impairment of the immune function (8–12). It has also been described in humans that leptin deficiency causes increased frequency and mortality of infections early in life (25). In conditions of nutritional deficit and reduced energy stores, leptin plays a crucial function that contributes to induce all adaptive mechanisms necessary to save energy, ensuring the correct function of vital organs such as heart, kidney and brain (26). Indeed, being inflammation and cell-mediated immune response energy consuming processes, the organism reduces activation and expansion of immune cells through leptin deficiency 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 sustain and promote CD4+ T cell-mediated autoimmune diseases (27). To evaluate whether leptin plays a role in the immunosuppression of malnutrition in humans, Palacio et al studied children with protein-calorie malnutrition, who had slightly decreased fat and significantly lower leptin levels. On admission, there was no correlation between body fat and leptin, reflecting the acute suppressive effects of malnutri-

**Fig. 3. Significant negative correlation between logarithm of leptin and PMN percentage.**
tion. 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 gamma and TNF alpha increased, whereas production of IL-4 decreased. These changes are similar, but not identical with the finding of leptin treatment in ob/ob and starved mice (28). The high leptin levels signal the presence of sufficient energy stores to the sites in the central nervous system, which respond by reducing appetite and increasing energy expenditure, preventing severe obesity (29). Therefore, leptin signals the nutritional status from the periphery to the area of the brain involved in the homeostasis of energy balance (29). The increased levels of leptin in hemodialysis patients are not only due to retention of the hormone, but probably due to increased production. Anorexia of hemodialysis patients has been attributed to the increased leptin levels, even if this is largely a hypothesis (30). 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 due to microinflammation caused by hemodialysis and the inflammatory stimuli 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 (31–33). We showed a significant positive correlation between serum leptin and body body mass index. More recent studies in long term dialysis patients suggest a paradoxically negative association between higher serum leptin and improved markers of nutritional status (32, 33). This finding is consistent with the theory of reverse epidemiology for leptin (32, 33). Indeed, leptin, has been reported to be a negative acute phase reactant in ESRD patients (29). Thus while an increased neutrophil count and reduced lymphocyte counts are independent predictors of increased mortality risk in hemodialysis patients, and while our study showed positive association of serum leptin to lymphocytes, and inverse correlation of serum leptin to PMNs, we can conclude that leptin has a protective role in decreasing mortality in hemodialysis patients and therefore serum leptin has a reverse epidemiology role by maintaining immune system in hemodialysis.

References


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