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ORIGINAL ARTICLE

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Relationship of leptin and insulin-like growth factor I to nutritional status in hemodialyzed children

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Abstract Malnutrition is prevalent in patients with end-stage renal disease (ESRD). Elevated serum leptin levels were thought to contribute to the anorexia and poor nutrition in renal failure. However, studies of the relationship between nutritional status and leptin concentration in chronic renal failure have yielded conflicting results. Plasma insulin-like growth factor I (IGF-I) level has been used as an indicator of nutritional status in patients with renal failure. The relationship between leptin and IGF-I is controversial. The present study was conducted with the aim of assessing the relationship between nutritional status, hyperleptinemia, and serum IGF-I. Seventeen ESRD patients (8 male, 9 female), aged 8–18 years (mean 15.3±3.3 years) and undergoing standard hemodialysis for 58.8±23.1 months were enrolled. Nine age-matched healthy children served as controls. In all patients, energy and protein intakes were 40–70 kcal/kg per day and 1–1.54 g/kg per day, respectively. Predialysis serum leptin and IGF-I levels were measured by radioimmunoassay. Body mass index was decreased in 13 (76%) patients. Triceps skinfold thickness (TST) was reduced (below the 5th percentile) in 7 (41%), whereas mid arm circumference and mid arm muscle circumference were reduced in 14 (82.5%) and 13 (76.5%), respectively. The median serum leptin level was significantly higher in patients than in controls [13.7 interquartile range (IQR) 30.50 pg/ml vs. 6.50 IQR 8.65 pg/ml, P=0.01]. The median serum IGF-I level was lower in the patients (205.1 ng/ml IQR 194.4 ng/l) than controls (418.0 ng/l IQR 310.5 ng/ml) (P=0.01). IGF-I levels were more decreased in patients with severe malnutrition, defined according to TST (145.0 ng/ml IQR 125.5 ng/l) than patients without malnutrition (301.2 ng/l IQR 218.8 ng/ml) (P=0.03) and healthy children (P=0.002). Although statistically not significant, IGF-I levels tended to be decreased, while leptin levels were increased. The median plasma insulin concentration was 15 μU/ml (1.63–45.80) and did not correlate with leptin and IGF-I levels. In conclusion, the results of this study confirm the presence of high circulating plasma leptin levels, which may be one of the many factors involved in the pathogenesis of the malnutrition in children on hemodialysis.

Keywords Nutritional assessment · Leptin · Insulin-like growth factor-I · Hemodialysis

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Introduction

Protein energy malnutrition (PEM) is prevalent in hemodialysis patients [1]. It is the consequence of inadequate nutritional intake due to a decreased appetite (anorexia) that can be due to a variety of causes [1]. The *ob* gene protein, leptin, which is produced by adipose tissue, is thought to be involved in regulating body fat by influencing food intake [2]. It has been reported that serum leptin levels are inappropriately elevated in children with chronic renal failure (CRF) and it has been suggested that the percentage of body fat was the main determinant [3]. In many reports, leptin has been shown to be closely related to body fat content in patients with CRF [4]. Since leptin is thought to be an inhibitor of appetite

by affecting the neuropeptide Y and/or melanocortin systems [5], it has been speculated that elevated serum leptin levels could contribute to anorexia and poor nutrition in patients with renal failure [4]. Hyperleptinemia in CRF is not well understood, although there is evidence that decreased renal elimination, inflammation, and hyperinsulinemia may contribute to raised leptin levels [6, 7]. A positive correlation between serum leptin and insulin levels in CRF has been documented in humans, suggesting that insulin resistance and hyperinsulinemia might contribute to hyperleptinemia in patients with end-stage renal disease (ESRD) [8].

Insulin-like growth factor I (IGF-I) has proved to be a sensitive marker of malnutrition. IGF-I synthesis is influenced by hormonal and nutritional factors [9, 10, 11, 12, 13]. The physiological relationship between leptin and IGF-I is poorly understood. Fontan et al. [14] suggested that leptin could primarily regulate IGF-I secretion. However, it has also been shown that growth hormone and IGF-I can modify serum leptin levels in ESRD [15]. The purpose of this study was to investigate the relationship between serum leptin, IGF-I, and nutritional status in children undergoing hemodialysis.

Materials and methods

Patients and controls

Seventeen children were investigated. There were 8 boys and 9 girls between 8 and 18 years of age (mean 15.3±3.3 years) undergoing chronic hemodialysis with 3–4 h hemodialysis sessions per week for a mean of 58.8±23.1 months. The median Kt/V ratio, which was used to estimate the dialysis adequacy, was 1.9 (1.4–2.5). Renal failure was due to glomerulonephritis in 11, reflux nephropathy in 4, and renal dysplasia in 2. Hollow-fiber dialyzers with cuprophane membranes were used. All patients were free from acute illness in the previous 3 months and none were receiving corticosteroids. Diabetic subjects were excluded. We recommended the dietary standards of FAQ/WHO Expert Committee for all patients [16]. The dietary intake of protein and calories was calculated from 4-day dietary diaries. In all patients, energy and protein intakes were between 40 and 70 kcal/kg per day and 1 and 1.54 g/kg per day, respectively. None of the subjects was given growth hormone therapy. Fifteen patients had been receiving erythropoietin treatment for at least 16 weeks before the study. Nine healthy children (5 boys, 4 girls, mean age 12.5±2.6 years) served as the control group.

Anthropometric evaluation

Anthropometric indices included: height (Ht), dry body weight (Wt), triceps skinfold thickness (TST), and mid-arm circumference (MAC). All measurements were taken by the same observer using a Harpenden caliper and a stadiometer. Mid-arm muscle circumference (MAMC) was calculated from MAC and TST, where MAMC=MAC–[3.14xTST(cm)]. Body mass index (BMI) was determined by dividing the Wt in kilograms by the square of the Ht in meters. We used percentile charts to determine the anthropometric measurements [17]. Based on Jacob et al. [9], severe malnutrition was defined with anthropometric measurements (TST, MAC, and MAMC) that were equal or less than the 5th percentile (depletion), whilst those between the 5th and 25th percentiles reflected undernutrition. For BMI, values less than 18.5 were considered as malnutrition, regardless of severity. However, we preferred TST to determine fat tissue, since it has been reported that plasma leptin is closely related to adipose tissue in patients with CRF [3].

Biochemical measurements

Predialysis blood samples were drawn without anticoagulation from non-fasting patients and controls in the morning before the dialysis and stored at −20°C until assayed. Serum concentrations of blood urea nitrogen (BUN), creatinine, total protein, albumin, and transferrin were measured in all patients. Quantitative C-reactive protein (CRP) levels were used for determination of chronic inflammation. Fasting insulin levels were measured by immunoradiometric assay. The serum concentration of IGF-I was measured by radioimmunoassay with a polyclonal rabbit anti-human antibody after extraction with acid-ethanol as previously described [18]. Intra- and inter-assay coefficients of variation were 6.5% and 15.7%, respectively. Serum leptin was measured by immunoradiometric assay (IRMA) (DSL Active Human Leptin IRMA kit, DSL-23100, USA). The specificity of both tests was 98%.

Statistical analysis

Results were expressed as median (minimum to maximum) for data not showing a normal distribution and as mean±SD (standard deviation) for data showing a normal distribution. Since the distributions of leptin and IGF-I were not normal, summary statistics were presented as medians and interquartile ranges (IQR) and non-parametric tests were performed. Comparisons of medians among the groups were performed by Kruskal-Wallis test. Whenever the P value was found to be significant, pairwise group comparisons were performed by Mann-Whitney U test. Correlation coefficients between variables were calculated by Spearman nonparametric correlation analysis. Statistical significance was assigned to P values lower than 0.05.

Results

Biochemical parameters

Median BUN and creatinine levels were 78 mg/dl (52–104) and 9 mg/dl (5.5–12.5), respectively. Median total protein and albumin levels were within normal limits [6.5 g/dl (5.5–7.4) and 4.2 g/dl (3.5–4.8), respectively]. The median serum transferrin level was 196 mg/dl (88–302) (normal >150 mg/dl). The median serum CRP level was 0.3 mg/dl (0.1–0.4) (normal <0.8).

Nutritional status

Anthropometric measurements of the patients were somewhat decreased compared with healthy controls (Table 1). BMI was decreased in 13 (76%) patients. TST was below the 25th percentile in 9 (53%) patients. MAC and MAMC were below the 25th percentile in 16 (94%) and 17 (100%) patients, respectively. TST was reduced (below the 5th percentile) in 7 (41%) patients, whereas MAC and MAMC were reduced in 14 (82.5%) and 13 (76.5%) patients, respectively. There were no significant correlations of serum transferrin, total protein, and albumin with anthropometric parameters.

Table 1 Anthropometric measures in hemodialysis patients and healthy controls (*BMI* body mass index, *TST* triceps skinfold thickness, *MAC* mid arm circumference, *MAMC* mid arm muscle circumference)

Anthropometric measurement	Hemodialysis patients Mean±SD	Healthy controls Mean±SD
BMI (kg/m^2)	17.1±1.6	19.4±3.6
TST (mm)	10.2±4.9	15.3±4.3
MAC (cm)	19.6±2.4	20.9±4.5
MAMC (cm)	16.4±1.8	20.5±2.0

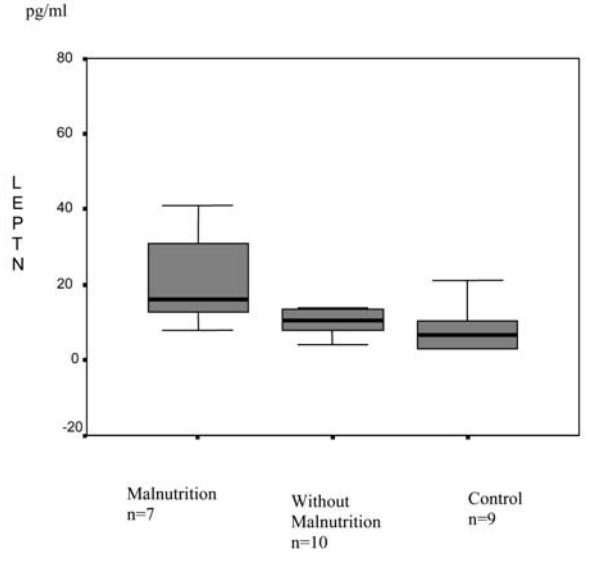

Fig. 1 Serum leptin levels in patients and controls according to triceps skinfold thickness

Leptin and IGF-I

The median serum leptin level was significantly higher in patients than controls (13.7 IQR 30.50 pg/ml vs. 6.50 IQR 8.65 pg/ml, P=0.01). Furthermore, in patients with severe malnutrition, leptin levels were significantly higher than in controls (16.0 IQR 29.0 pg/ml vs. 6.50 IQR 8.65 pg/ml (P=0.01) (Table 2, Fig. 1). However, the median leptin level was not statistically different between patients with severe malnutrition and those without malnutrition (P>0.05). Although statistically not significant, we found an inverse relationship between leptin and TST (r=–0.43, P=0.08).

The median serum IGF-1 level was lower in the patients (205.1 IQR 194.4 ng/l) than controls (418.0 IQR 310.5 ng/l) (P=0.01), which reflected the IGF-I levels of the same age group in the Turkish population [19]. Furthermore, IGF-I levels were more decreased in patients with severe malnutrition defined according to TST (145.0 IQR 125.5 ng/l) than in patients without malnutrition (301.2 IQR 218.8 ng/l) (P=0.03) and healthy children (P=0.002). In contrast, there were no significant differences between the IGF-I levels in patients below the 25th percentile according to TST and those in healthy controls (P=0.28). Leptin levels tended to be increased, while IGF-I levels were decreased (r=–0.07, P=0.8).

The median plasma insulin concentration was 15 μU/ml (1.63–45.80). Plasma insulin levels did not correlate with leptin and IGF-I levels (r=0.12, P>0.05 and r=0.15, P>0.05, respectively).

Discussion

The present study documented an elevated plasma leptin concentration in children undergoing hemodialysis. It has been demonstrated in previous studies that the concentration of serum leptin is closely related to body fat content in humans with CRF [4, 7]. Daschner et al. [3] showed that only the percentage of body fat assessed from skinfold measurement and glomerular filtration rate contributed significantly to the variability of serum leptin levels using multiple stepwise regression analysis containing gender, age, pubertal stage, fasting glucose concentration, serum albumin, protein, and transferrin levels. Based on these findings, we used TST for the determination of nutritional status and found a tendency for an inverse relationship between these parameters and plasma leptin levels.

Very little is known about the physiological actions of leptin in humans, but administration of recombinant leptin to *ob/ob* mice, which have a genetic defect in leptin production, reduces food intake and increases energy expenditure [20]. In children with CRF, an inverse linear correlation between leptin levels and energy intake expressed as the percentage of Recommended Dietary Allowance (RDA) has been reported [3]. Elevated serum leptin levels were thought to contribute to the development of anorexia and poor nutrition in patients with renal failure [4]. However, subsequent studies investigating the

Table 2 Serum median insulin-like growth factor I (IGF-I) and leptin levels in hemodialysis patients and healthy controls (*IQR* interquartile range)

	Patients			Controls Median IQR (n=9)
	Total Median IQR (n=17)	Severe malnutrition Median IQR (n=7)	Without malnutrition Median IQR (n=10)	
IGF-I (ng/ml)	205.1 IQR 194.4*[1]	145.0 IQR 125.5*[2]	301.2 IQR 218.8	418.0 IQR 310.5
Leptin (pg/ml)	13.7 IQR 30.5*[3]	16.0 IQR 29.0*[4]	10.6 IQR 6.7	6.50 IQR 8.65

*[1] P=0.01 patients vs. controls; *[2] P=0.03 and P=0.002 severe malnutrition vs. without malnutrition and controls, respectively; *[3] P=0.01 patients vs. controls; *[4] P=0.01 severe malnutrition vs. controls

relationship between nutritional status and leptin concentration in CRF have yielded conflicting results, showing that the issue is certainly more complex than initially thought [21]. In the present study, although there was a tendency to higher leptin levels in severely malnourished patients than in those without malnutrition, there was no correlation with the TST values. The lack of a statistically significant difference in plasma leptin levels between patients with severe malnutrition and those without malnutrition, as well as a lack of correlation between plasma leptin and TST, fail to support the first-line role of hyperleptinemia in the genesis of PEM.

Impaired peripheral sensitivity to insulin and hyperinsulinemia are well-recognized features of CRF [22]. However, our results showed that markedly elevated concentrations of serum leptin in relation to the percentage of body fat were associated with normal plasma insulin levels. This may indicate that high serum leptin levels inhibit pancreatic beta-cell function in CRF. Indeed, leptin receptors (Ob-Rb) were found in the pancreas and leptin inhibits insulin secretion in mice in a dose-dependent manner [22].

The plasma IGF-I level has been used as an indicator of nutritional status in patients with renal failure. This correlates significantly with anthropometric markers of malnutrition and has been shown to be a good indicator of malnutrition in children and adults [11, 12, 13]. The relationship between leptin and IGF-I is controversial. Leptin was thought primarily to regulate IGF-I secretion, the inverse relationship representing a feedback mechanism [14]. However Dagogo-Jack et al. [23] showed that basal plasma leptin and IGF-I levels were not correlated. Chronic administration of recombinant human IGF-I was associated with an early and sustained decrease in plasma leptin levels. This suggested that IGF-I might have an inhibitory effect on leptin secretion in humans by directly reducing leptin release from adipocytes [23, 24]. Iglesias et al. [25] confirmed the presence of high circulating plasma leptin in dialysis patients and showed that plasma leptin levels were further increased by exogenous administration of recombinant human growth hormone (rhGH). This suggested that the increase in plasma leptin after rhGH therapy might be related to the rhGH-induced changes in insulin in patients. In our study, IGF-I levels of the patients were lower than those of healthy children. Furthermore, serum IGF-I levels were low in patients with depleted (below the 5th percentile) body fat stores compared with patients without depletion and healthy controls. Although statistically not significant, probably due to an insufficient number of severely malnourished patients, our observation that there was an inverse relationship between serum leptin and IGF-I levels might indicate that decreased IGF-I levels contribute to elevated leptin.

Elevated serum leptin levels in renal failure might be secondary to chronic inflammation. There is evidence that leptin secretion is regulated by tumor necrosis factor-α. It has been shown that increased CRP levels, which indicate an ongoing inflammatory response, stimulate adipose tissue *ob* gene expression in patients with CRF [26]. However, in our patients, normal CRP levels suggested that hyperleptinemia was not a consequence of chronic inflammation.

In conclusion, the results of this study confirm the presence of high circulating plasma leptin levels, which may be one of the many factors involved in the pathogenesis of the malnutrition in children on hemodialysis.

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