Original paper

A positive correlation of serum homocysteine with leptin in maintenance hemodialysis patients

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Abstract

Introduction: Serum leptin is cleared principally by the kidneys and serum leptin concentrations are increased in patients with chronic renal failure and those undergoing a maintenance dialysis. Mild-to-moderate elevations in plasma total homocysteine (Hcy) levels are also observed in the great majority of patients with end-stage renal disease who are undergoing a dialysis. A paradoxically inverse association between higher serum leptin and improved markers of the nutritional status was shown. Based on the above mentioned data regarding the associations of homocysteine and leptin with the nutritional status, we tested the hypothesis that the concentration of serum homocysteine and leptin in hemodialysis patients may have an association.
Material and methods: In a cross-sectional analysis on 36 (F=15, M=21) stable hemodialysis (HD) patients, consisting of 25 non-diabetic HD patients and 11 diabetics, serum homocysteine and serum leptin were measured.
Results: In this study a significant positive correlation of serum leptin with homocysteine and a significant positive correlation of homocysteine with the body mass index (BMI) and also a significant positive correlation of leptin with BMI were found.
Conclusions: The positive correlations of serum homocysteine with leptin and also with BMI show its good correlation with the nutritional status of regular hemodialysis patients.

Key words: leptin, homocysteine, maintenance hemodialysis patients, body mass index, malnutrition-inflammation cachexia syndrome.

Introduction

Homocysteine (Hcy) is a sulphur amino acid formed from methionine during transmethylation, and is either salvaged to methionine by a folate- and cobalamin-dependent re-methylation reaction or directed toward degradation by the vitamin B_6-dependent enzyme cystathionine β-synthase [1, 2]. Large studies have demonstrated that moderate hyperhomocysteinaemia is an independent risk factor for premature atherosclerosis and cardiovascular disease [3]. Mild-to-moderate elevations in plasma total homocysteine (Hcy) levels are observed in the great majority (>85%) of patients with end-stage renal disease who are undergoing a maintenance dialysis [3, 4]. In maintenance hemodialysis patients (MHDPs), the association between Hcy and clinical outcome is inconsistent and even

paradoxical. Some studies have shown a poor outcome in MHD patients with hyperhomocysteinemia. Recent studies have suggested that a decreased, not an increased, Hcy concentration is related to a higher prevalence of cardiovascular disease and poor outcome in these individuals [4-6]. Indeed plasma levels of total homocysteine are influenced by the nutritional status in patients with chronic kidney disease [7, 8]. More recent studies showed that an increased level of total plasma homocysteine is a risk factor for poor cardiovascular outcome in the general population. However, a decreased, rather than an increased, Hcy concentration may predict poor outcome in maintenance hemodialysis (MHD) patients, a phenomenon referred to as reverse epidemiology [9]. This seems to be due to the association between a low Hcy and protein-energy malnutrition, which is per se a known risk factor for poor clinical outcome in dialysis patients [6, 10]. This recently described paradoxical association between tHcy and clinical outcome in dialysis patients has now been referred to as a possible component of the reversal of the cardiovascular risks [11-13]. It is believed that both inflammation and protein-energy malnutrition, each independently or together as the "malnutrition-inflammation complex syndrome" are responsible for this condition [13-17]. Studies have demonstrated that leptin is cleared principally by the kidney and serum leptin concentrations are increased in patients with chronic renal failure and those undergoing a maintenance dialysis [18, 19]. Studies in maintenance dialysis patients also suggest a paradoxically inverse association between higher serum leptin and improved markers of the nutritional status [20, 21]. Based on the above mentioned data regarding the associations of homocysteine and leptin with the nutritional status, we tested the hypothesis that a high concentration of serum homocysteine and leptin in hemodialysis patients may have a correlation with each other. Therefore in a group of hemodialysis patients containing diabetics and non-diabetics, we considered this correlation to better find the association of homocysteine and leptin with various nutritional parameters in MHDPs.

Material and methods

Patients

This cross-sectional study was conducted on patients with end-stage renal disease (ESRD), who were undergoing a 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 D_3 (Calcitriol; Rocaltrol) (Roche Hexagon; Roche Laboratories Inc., New Jersey, USA), calcium carbonate capsule, and Rena-Gel (sevelamer; Genzyme Europe B.V.; United Kingdom/Ireland) tablets at various doses. According to the severity of anemia, patients were prescribed intravenous iron therapy with Iron Sucrose (Venofer) (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; Janssen-Cilag; CILAG-AG International 6300 Zug/Switzerland) after each dialysis session. Exclusion criteria were active or chronic infection and using NSAID or ACE inhibitor drugs. The study was done in the hemodialysis section of the Hajar Medical Educational & Therapeutic Center of Shahrekord University of Medical Sciences in Shahrekord of Iran.

Laboratory methods

Serum homocysteine (total) and serum leptin were measured as follows. Blood samples were drawn after an overnight fast. All blood samples were centrifuged within 15 min of venepuncture, and were measured by the enzyme-linked immunosorbent assay (ELISA) method using DRG kits (DRG Diagnostics, Berlin, Germany). Serum leptin has a normal range of 3.84 (±1.79) for males and 7.36 (±3.73) ng/ml for females and serum total homocysteine has a normal range of 25-125 µmol/L. Also peripheral venous blood samples were collected after an overnight fast, for the hemoglobin level (using Sysmex-KX-21N Cell counter; Sysmex Corporation; Mundelein, Illinois, Sysmex America, Inc.) and a biochemical analysis including serum predialysis creatinine (Creat), post and predialysis blood urea nitrogen (BUN), albumin (Alb) as well as serum C-reactive protein (CRP). For patients levels of serum ferritin by the radio immune assay method (RIA), were measured using standard kits. Body mass index (BMI) calculated using the standard formula (postdialyzed weight in kilograms/height in square meters; kg/m^2). For the efficacy of hemodialysis the urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data. Duration and the amount of sessions of hemodialysis treatment were calculated from the patients' records. The duration of each hemodialysis session was 4 hours.

Statistical analysis

Results are expressed as the mean ±SD and median values. The comparison between the groups was done using the Student's t-test. Statistical correlations were assessed using the partial correlation test. The statistical analysis was performed on total hemodialysis (HD), females,

Table I. Patients' data

Total patients N=36	**Minimum**	**Maximum**	**Mean ±SD**	**Median**
Age [years]	27	75	53±15.8	43
DH* [months]	6	24	14.5±6	36
Dialysis sessions	54	216	123±54	156
URR [%]	39	75	53.5±9.8	57.5
Leptin [ng/ml]	0.10	73	9.4±14	5.75
Hgb [g/dl]	5	13	9±2	9
Homocysteine [µmol/L]	1.2	12.8	5±2.4	4.5
Ferritin [ng/dl]	35	1250	519±299	426
Alb [g/dl]	2.4	4.8	3.8±0.5	3.95
CRP [mg/l]	3	40	8.7±6.7	8
BMI [kg/m^2]	16	34	21.8±4.5	21.5
Non-diabetic patients N=25	**Minimum**	**Maximum**	**Mean±SD**	**Median**
Age [years]	16	80	44±17	41
DH* [months]	2	156	40±40.8	22
Dialysis sessions	36	1584	370±452	156
URR [%]	60	76	61±7.5	60
Leptin [ng/ml]	0.10	52	7.6±10.5	4
Hgb [g/dl]	5	12	8.5±2	9
Homocysteine [µmol/L]	1.2	9.7	5±2	5.6
Ferritin [ng/dl]	170	1250	576±282	483
Alb [g/dl]	2.4	4.7	3.8±0.5	4
CRP [mg/l]	2	20	7.4±3.8	6
BMI [kg/m^2]	16	33	21±4.6	19
Diabetic patients N=11	**Minimum**	**Maximum**	**Mean±SD**	**Median**
Age [years]	27	75	53±15.8	55
DH* [months]	6	24	14.5±6	12
Dialysis sessions	54	216	123±54	108
URR [%]	39	75	53.5±9.8	54
Leptin [ng/ml]	0.2	73	13.6±20	8.3
Hgb [g/dl]	0.5	13	10±2	10
Homocysteine [µmol/L]	2.8	12.8	5±3	4.3
Ferritin [ng/dl]	35	1000	388±308	278
Alb [g/dl]	3	4.8	3.8±0.5	3.9
CRP [mg/l]	4	40	12±10	10
BMI [kg/m^2]	20	34	23±4	23

**duration of hemodialysis*

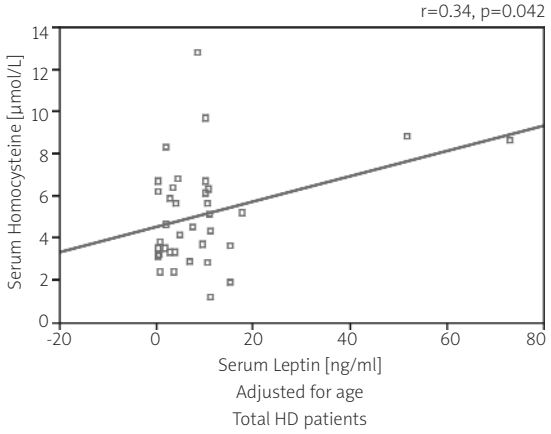

Figure 1. A significant positive correlation of serum leptin with serum homocysteine

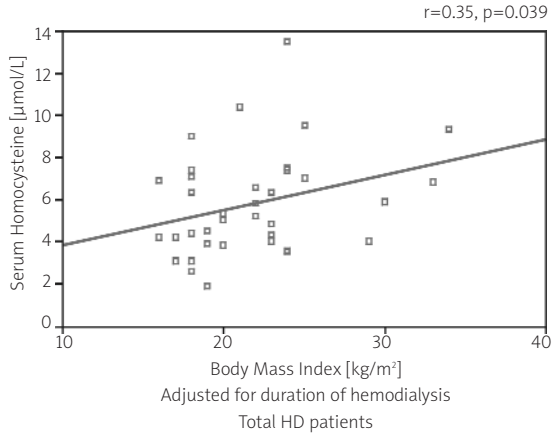

Figure 2. A significant positive correlation of serum homocysteine with BMI

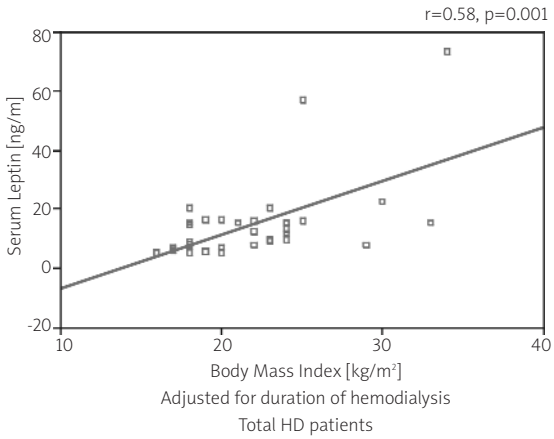

Figure 3. A significant positive correlation of serum Leptin with BMI

males, diabetics and non diabetics populations separately. All statistical analyses were performed using SPSS (version 11.5.00) (SPSS Inc., Chicago, IL). The statistical significance was determined at a p-value below than 0.05.

Results

The study patients were 36 (F=15, M=21), consisting of 25 (F=11, M=14) non-diabetic HD patients and 11 (F=4, M=7) diabetic HD patients. Table I shows the patients' data. The mean patient's age was 53 (±15.8) years. The value of serum homocysteine of all HD patients was 5 (±2.4) µmol/L. The values of serum Hcy of diabetic and nondiabetic-dialysis patients were 5 (±3) and 5 (±2) µmol/L, respectively. The value of serum leptin of all HD patients was 9.4 (±14) ng/ml. The values of serum leptin of diabetic and non-diabetic-dialysis patients were 13.6 (±20) and 7.6 (±10.5) ng/ml, respectively. There were no significant differences of serum leptin between diabetic and non-diabetics or male and female HD patients also no significant differences of serum homocysteine between diabetic and non--diabetics or male and female HD patients were found. In all HD patients a significant positive correlation of serum leptin with serum homocysteine ($r=0.34$, $p=0.042$; Figure 1) (adjusted for age) was seen. In this group also a significant positive correlation of serum homocysteine with BMI ($r=0.35$, $p=0.039$; Figure 2) (adjusted for duration of dialysis) was seen, too. Moreover, in this group a significant positive correlation of serum leptin with BMI ($r=0.58$, $p<0.001$; Figure 3) (adjusted for age) was seen.

Discussion

In this study a significant positive correlation of serum leptin with serum homocysteine and a significant positive correlation of serum homocysteine with BMI were seen. Also a significant positive correlation of serum leptin with BMI was found too. To examine the association between the homocysteine level and markers of malnutrition--inflammation complex syndrome and 12-months prospective hospitalization and mortality in 367 MHDPs, aged 54.5±14.7 years, Kalantar-Zadeh et al. found weak to moderate but statistically significant correlations of hyperhomocysteinemia with some laboratory markers of nutrition (serum albumin, prealbumin, creatinine, and urea nitrogen) but no significant correlation with serum C-reactive protein existed [10]. They found that the hospitalization rates were significantly higher in patients with lower Hcy levels and mortality rate in the lowest Hcy quartile was significantly higher compared with other three quartiles. They concluded that Hcy may be a more exclusive nutritional marker in MHD patients with no association with inflammatory measures [10]. In a study conducted by Suliman et al. on a cohort of 250 patients with chronic kidney disease (CKD) which starting a renal replacement therapy to assess the overall mortality in relation to basal tHcy level followed the patients during a 4-year period. They found that ninety-three patients (37%) with signs of

inflammation (CRP >or =1 mg/dL) had significantly lower levels of tHcy and serum Alb than 157 noninflamed patients. Homocysteine levels correlated positively with serum Alb levels and negatively with CRP levels and other inflammation markers. They showed that the presence of both inflammation and malnutrition was associated with lower homocysteine levels than when malnutrition was present without inflammation. They also showed that that serum Alb and CRP levels were independently associated with Hcy levels after adjustment for other variables. The serum Hcy level was significantly greater in survivors than nonsurvivors, and a greater Hcy level was associated with better survival, moreover, they concluded that plasma Hcy level was lower in patients with inflammation and inflammation may contribute to the reverse association between the Hcy level and mortality in patients with CKD starting a renal replacement therapy [8]. Recent studies in maintenance dialysis patients suggest a paradoxically inverse association between higher serum leptin and improved markers of nutritional status [20, 21], a finding that is consistent with the theory of reverse epidemiology [16]. Indeed, leptin, similar to serum albumin, has been reported to be a negative acute phase reactant in end-stage renal failure patients [21, 22].

Conclusions

The positive correlations of serum homocysteine with leptin and also with BMI show its well association with nutritional status of regular hemodialysis patients.

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