

THE CORRELATIONS BETWEEN PLASMA LEPTIN AND THE CONCENTRATIONS OF PROINFLAMMATORY CYTOKINES IN PATIENTS WITH TYPE II DIABETES MELLITUS IN MAINTENANCE HEMODIALYSIS

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Abstract

The goal of the study was to assess the serum level of leptin and to establish if there is a relationship between leptin and proinflammatory cytokines in diabetic and non-diabetic patients submitted to chronic hemodialysis.

The study included 20 type II diabetic patients and 15 non-diabetic patients in maintenance hemodialysis. A group of 30 healthy age-matched subjects were used as control. The serum levels of leptin and inflammatory cytokines were determined: IL-6 (Interleukin 6), IL-8 (Interleukin 8), TNF- α (Tumor necrosis factor) before and after hemodialysis.

The diabetic female patients on dialysis have leptin, TNF- α , IL-8 levels significantly higher than in control group ($p < 0.0001$). Leptin did not show critical differences in the three male groups ($p > 0.05$). A negative correlation was detected only in diabetic female patients between serum ferritin and serum leptin (Pearson index -0.6062) and a positive correlation with BMI (Body mass index), (Pearson index 0.6657).

Serum leptin levels in hemodialysed patients with type II diabetes mellitus do not differ significantly from those of hemodialysed non-diabetic patients.

Rezumat

Scopul studiului a fost de a evalua nivelul seric al leptinei și de a stabili dacă există o relație între leptină și citokinele proinflamatorii la pacienții cu diabet zaharat și fără diabet zaharat, hemodializați cronic.

Studiul a inclus 20 de pacienți cu diabet zaharat tip II și 15 pacienți non diabetici hemodializați. Un grup de 30 de voluntari sănătoși cu vârste asemănătoare a fost folosit ca

și lot martor. A fost determinat nivelul seric al leptinei și citokinelor inflamatorii: IL-6 (Interleukina 6), IL-8 (Interleukina 8), TNF- α (Factorul alfa de necroză tumorală), atât înainte, cât și după hemodializă.

Pacienții diabetici de sex feminin hemodializați au valori ale leptinei, TNF- α și IL-8 semnificativ mai mari decât ale lotului martor ($p < 0,0001$). Leptina nu a prezentat diferențe semnificative la pacienții de sex masculin din cele trei loturi ($p > 0,05$). O corelație negativă a fost detectată doar la pacienții cu diabet zaharat de sex feminin între valorile feritinei serice și cele ale leptinei serice (indicele Pearson -0.6062), iar o corelație pozitivă a fost stabilită cu IMC (Index de masă corporală), (indicele Pearson 0.6657).

Valorile serice ale leptinei la pacienții hemodializați cu diabet zaharat de tip II nu diferă în mod semnificativ față de cele ale pacienților hemodializați non-diabetici.

Keywords: Leptin, TNF- α , IL-6, IL-8, hemodialysis, diabetes mellitus

Introduction

Leptin was identified and cloned in 1994 [27]. It is a 167-amino-acid protein mainly produced by adipocytes. Recent studies showed that leptin is also secreted in other tissues such as stomach, skeletal muscles, ovaries, liver and heart [28].

The hormone also stimulates the production of proinflammatory cytokines, vascular inflammation, generation and accumulation of reactive oxygen species and vascular smooth muscle hypertrophy, which may contribute to the pathogenesis of hypertension, atherosclerosis, coronary heart disease and type 2 diabetes mellitus [22, 4, 5, 26, 20, 3].

End-stage renal disease (ESRD) is associated with anorexia, malnutrition, inflammation, oxidative stress, increased production and release of proinflammatory cytokines [18, 11, 12].

TNF- α (tumor necrosis factor alpha) an inflammatory cytokine can also affect energy metabolism. It can promote leptin production and alter lipid and glucose metabolism and reduce insulin sensitivity in white adipose tissue (16). It was described that in chronic renal failure patients serum leptin levels are elevated compared to the control group [14, 23].

The aim of the study was to test the serum level of leptin and to establish if there is a correlation between leptin and proinflammatory cytokines in diabetic and non-diabetic patients submitted to chronic hemodialysis.

Material and methods

Patients

The study was carried out on twenty type II diabetic patients, twelve females (64.42 ± 8.08 years) and eight males (aged 64.25 ± 5.99 years) in

maintenance hemodialysis. Another group of fifteen non-diabetic patients, six females and nine males, aged 65.66 ± 8.89 , respectively 55.90 ± 10.04 years) on dialysis were included. A group of thirty healthy age-matched subjects (15 males and 15 females, aged 50.53 ± 13.35 , respectively 49.67 ± 10.64 years) were used as control.

All patients and controls were submitted to anamnesis, clinical examination and anthropometric measures in order to determine the body mass index (BMI). Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters.

The duration of diabetes was 15.92 ± 6.44 years. Only patients with anuria were included in the study, which is considered diuresis lower than 250 mL in 24 hours.

Written informed consent was obtained from all participants, prior to enrolment approved by the institutional ethical committee. The blood samples were collected after overnight fasting. The blood was collected in vacuum tubes. Specimens were transported to the laboratory immediately after collection and centrifuged at 1500g for 10 min to separate serum.

For leptin serum concentrations we used an enzyme-linked immunosorbent assay (ELISA) DRG Diagnostic kit on ELISA Automat Adaltis.

Measurement of serum IL-6, IL-8, TNF- α were performed with a chemiluminescent sequential immunometric assay, Siemens kit on an Immulite 1000 analyzer.

Routine laboratory tests on automated analyzer using commercial kits also were assessed.

Statistical analysis

The data were expressed as mean \pm SD. P-values < 0.05 were considered as significant. Two-way ANOVA with Bonferroni post-test was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA www.graphpad.com)

Results and Discussion

General data – biochemical characteristics among the different investigated groups

General characteristics of diabetic and non-diabetic patients and comparison to controls (females and males) are summarized in Table I and Table II.

Table I
Biochemical characteristics of the female investigated groups

Characteristics	Control	Diabetic patients	Non-diabetic patients
Women (n)	15	12	6
Age (years)	50.53 ± 13.35	64.42 ± 8.08	65.66 ± 8.89
BMI (Kg/m ²)	22.34 ± 1.12 ^a	31.61 ± 6.29 ^b	23.61 ± 5.70 ^{a,b}
Creatinine (mg/dL)	0.85 ± 0.19 ^a	12.33 ± 18.33 ^c	6.82 ± 1.90 ^b
Urea (g/L)	0.29 ± 0.09 ^a	1.40 ± 0.18 ^b	1.15 ± 0.41 ^b
Ferritin (µg/L)	74.13 ± 18.00 ^a	500.25 ± 8.73 ^b	527.33 ± 20.65 ^c
Fibrinogen (g/L)	2.59 ± 0.36 ^a	3.79 ± 0.69 ^a	3.92 ± 0.52 ^a
Uric acid (mg/dL)	3.41 ± 0.77 ^a	6.61 ± 1.24 ^{a,b}	5.80 ± 1.58 ^a
Total proteins(g/L)	69.91 ± 5.22	66.63 ± 5.75	66.00 ± 6.23
Iron (µg/100mL)	88.45 ± 18.66 ^a	76.25 ± 25.31 ^b	58.17 ± 35.05 ^a
Leptin ng/mL	5.26 ± 1.67 ^a	16.55 ± 6.52 ^b	17.62 ± 8.19 ^b

^{a,b,c} Different superscripts in the same row indicate statistically significant differences ($p \leq 0.05$). Values with same superscript are not different (Two-way ANOVA - Bonferroni multiple comparisons)

Table II
Biochemical characteristics of the male investigated groups

Characteristics	Control group	Diabetic patients	Non-diabetic patients
Men (n)	15	8	9
Age (years)	49.67 ± 10.64	64.25 ± 5.99	55.90 ± 17.04
BMI (Kg/m ²)	23.98 ± 0.76 ^a	29.12 ± 4.49 ^a	22.59 ± 5.41 ^a
Creatinine (mg/dL)	0.85 ± 0.19 ^a	10.02 ± 2.60 ^b	8.61 ± 2.40 ^b
Urea (g/L)	0.29 ± 0.089 ^a	1.58 ± 0.35 ^b	1.25 ± 0.25 ^b
Ferritin (µg/L)	85.40 ± 19.19 ^a	365.00 ± 29.23 ^b	432.00 ± 73.68 ^c
Fibrinogen (g/L)	2.60 ± 0.4 ^a	3.88 ± 1.07 ^a	3.51 ± 0.73 ^a
Uric acid (mg/dL)	5.08 ± 0.93 ^a	6.41 ± 1.26 ^a	6.17 ± 0.68 ^a
Total proteins (g/L)	74.81 ± 3.62 ^a	68.16 ± 5.57 ^a	66.14 ± 4.03 ^a
Iron (µg/100mL)	97.03 ± 3.62 ^a	81.00 ± 31.03 ^b	97.00 ± 37.96 ^a
Leptin ng/mL	4.42 ± 1.42 ^a	9.73 ± 5.28 ^a	5.40 ± 5.21 ^a

^{a,b,c} Different superscripts in the same row indicate statistically significant differences ($p \leq 0.05$). Values with same superscript are not different (Two-way ANOVA - Bonferroni multiple comparisons)

Cytokine values

Cytokine values of diabetic and non-diabetic patients and comparison to controls (females and males) are summarized in Table III and Table IV.

Table III

Mean and standard deviation (SD) cytokine values of the female groups

Cytokine	Control group	Diabetic patients	Non-diabetic patients
TNF- α pg/mL	6.97 \pm 1.52 ^a	25.61 \pm 6.61 ^b	25.08 \pm 7.15 ^b
IL6 pg/mL	2.89 \pm 1.20 ^a	8.11 \pm 3.80 ^a	10.91 \pm 6.67 ^{a, b}
IL8 pg/mL	18.96 \pm 4.99 ^a	37.93 \pm 5.88 ^b	38.13 \pm 7.66 ^b

^{a,b} Different superscripts in the same row indicate statistically significant differences ($p \leq 0.05$). Values with same superscript are not different (Two-way ANOVA - Bonferroni multiple comparisons)

Table IV

Mean and standard deviation (SD) cytokine values of the male groups

Cytokine	Control group	Diabetic patients	Non-diabetic patients
TNF α pg/mL	6.79 \pm 1.42 ^a	27.20 \pm 4.19 ^b	24.95 \pm 3.80 ^b
IL6 pg/mL	2.72 \pm 1.65 ^a	11.95 \pm 5.41 ^b	7.81 \pm 5.73 ^{a, b}
IL8 pg/mL	17.22 \pm 6.64 ^a	41.20 \pm 11.53 ^b	30.84 \pm 6.08 ^c

^{a,b,c} Different superscripts in the same row indicate statistically significant differences ($p \leq 0.05$). Values with same superscript are not different (Two-way ANOVA - Bonferroni multiple comparisons)

In our study the diabetic female patients submitted to dialysis had leptin, TNF- α , IL-8 levels significantly higher than in control group ($p < 0.0001$), except the level of IL-6, where no significant difference was recorded (Table I and III). The levels of cytokines were significantly higher between control group and non-diabetic female patients. Between diabetic and non-diabetic female groups no significant differences were noted in all cytokines and leptin investigated ($p > 0.05$). When we compared the diabetic with non-diabetic male group, regarding cytokine level, no significant differences were recorded, except the level of IL-8 ($p < 0.01$). Also, the male diabetic patients had TNF- α , IL-6, IL-8 levels significantly higher than in control group ($p < 0.0001$). Between control and non-diabetic male groups, significant differences were noted in all cytokine levels investigated, except the level of IL-6 where $p > 0.05$ (Table IV).

In comparison with the control group, ferritin serum concentration in the two female patients studied groups were considerable significantly higher ($p < 0.0001$).

The levels of leptin did not show any significant differences between the male groups investigated ($p > 0.05$) (Table II).

Correlation between leptin and cytokines and some biochemical parameters in different investigated groups

After dividing the patients in diabetic and non-diabetic, in gender groups the correlation between leptin and biochemical parameters in each group was estimated.

A negative significant correlation between serum leptin and serum ferritin in diabetic female patients was found (Pearson index -0.6062). In the same group of patients leptin had a positive correlation with BMI (Pearson index 0.6657). The connection with other variables was not significant.

The correlation between leptin and BMI in non-diabetic female patients group was significant (Pearson index 0.8649).

Table V

Pearson correlation coefficient (R) of leptin with cytokines and some biochemical variables

Investigated group	Leptin	BMI	TNF- α	IL6	IL8	Ferritin	Fibrinogen	Creatinine
Diabetic females	R	0.6657	0.4728	-0.1706	-0.0237	-0.6062	-0.1864	0.3951
	P	0.0181*	0.1206	0.5961	0.9417	0.0367*	0.5620	0.2036
Diabetic males	R	-0.1493	-0.2571	-0.2722	-0.4476	0.2487	-0.0218	-0.2931
	P	0.7493	0.5668	0.5548	0.3140	0.5907	0.9630	0.5235
Non-diabetic females	R	0.8649	-0.1750	-0.3439	-0.4050	0.0060	0.2450	0.4551
	P	0.0262*	0.7402	0.5045	0.4257	0.9923	0.6398	0.3644
Non-diabetic males	R	0.4319	0.2732	-0.3823	0.2839	0.3746	-0.1675	0.3519
	P	0.2126	0.4450	0.2755	0.4266	0.2863	0.6436	0.3187

The relation between leptin and other biochemical parameters was insignificant in non-diabetic and diabetic male hemodialysis group (Table V).

In this study we compared the levels of serum leptin and cytokine in the group of patients and eventually find a correlation between them.

Recent papers indicate that adipose tissue secretes leptin, interleukins, tumor necrosis factor alpha which contribute to systemic inflammation in dialysis patients. Chronic inflammation is recognized as a complication of the dialysis [2].

In this study we found that serum leptin levels were significantly higher in the two groups of diabetic and non-diabetic female patients on

dialysis than in control group ($p < 0.0001$). No modifications were noted between diabetic and non-diabetic female patients on dialysis ($p > 0.05$). In the three male groups studied the serum leptin concentration had no major variations ($p > 0.05$). Our results are similar with other published results [10].

Lee *et al.* demonstrated that serum leptin levels were elevated in hemodialysis patients [13].

Leptin becomes elevated with renal impairment. This is probably due to a decrease in renal clearance which occurs in glomerular filtration followed by metabolic degradation in the renal tubular cells [6].

This is explained by the hyperleptinemia in renal failure despite a lower leptin gene (*ob* gene) expression [17].

The conclusion was that leptin plays an important role in the diabetic complication.

In our paper leptin levels were negatively correlated with ferritin in diabetics female patients on dialysis (Pearson index -0.6062) and with BMI a poor and positive correlation (Pearson index 0.6657). This is due to the fact that leptin behaves as an inverse acute-phase reactant during episodes of the acute-phase response. Our results are similar with other studies [7].

A correlation between ferritin and serum leptin in diabetic male patients was not found.

The results demonstrate a poor positive connection between serum leptin and BMI in non-diabetic female patients on dialysis (Pearson index $r = 0.8649$).

IL-6 is produced by immune cells, fibroblasts, endothelial cells, skeletal muscle and adipose tissue [15]. IL-6 is proatherogenic and associated with poor outcome of hemodialysis [24]. TNF- α and IL-6 inhibit lipoprotein lipase [8].

In our investigations there was no significant difference in the serum IL-6 levels of diabetic female patients and control groups ($p > 0.05$), but considerable higher in male diabetic patients ($p < 0.0001$).

Many studies have pointed out the role of TNF- α and IL-6 in insulin resistance, disturbed glucose tolerance and microangiopathy [9, 21].

In our paper, plasma TNF- α concentrations were found to be similar in diabetic and non-diabetic female and male patients, but in comparison with the control group they were significantly higher.

IL-8 is released mainly by the stimulated macrophages. IL-8 concentration were significantly higher in the diabetic female patients than in control group ($p < 0.0001$), but no modifications were observed between diabetic and non-diabetic female patients on dialysis ($p > 0.05$).

In the diabetic patients with chronic renal failure a progressive elevation of urea appears. A role of urea in the release of leptin was recently described [1]. The level of urea in our group of patients was high. After dialysis, the concentration of urea decreased without the reduction of the leptin. This is an argument that the stimulation of leptin secretion is not the result of unique urea action.

Studies of the correlation between insulin and leptin were in the last time promoted. The results outlined two opposite conceptions. Some researchers proved that leptin worsened the evolution of diabetes by reducing insulin sensitivity [1, 25] and others that leptin improves glucose tolerance [19].

These are issues to be also solved in the future.

Conclusions

Our results showed that leptin levels were negatively correlated with ferritin in diabetic female patients on dialysis. We found that serum leptin concentrations were considerably higher in the groups of diabetic and non-diabetic female patients on dialysis compared to the control group. The results demonstrate a slight positive correlation between serum leptin and BMI in non-diabetic female patients on dialysis. No significant relationship between serum leptin and pro-inflammatory cytokines was found.

Other studies are needed to be extended for a better understanding of the role of pro-inflammatory cytokines in patients with diabetes submitted to maintenance hemodialysis in order to prevent further deterioration in renal function and the need for renal transplant.

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