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THE LEVELS OF SERUM BIOMARKERS OF INFLAMMATION IN HEMODIALYSIS PATIENTS

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Abstract

Leptin controls metabolism, energy balance and can also intensify inflammation by inducing mononuclear cell activation and proliferation. It realizes this last effect by stimulating the production of pro-inflammatory cytokines such as interleukine-6 (IL-6), tumour necrosis factor α (TNF- α), interleukine-8 (IL-8). The objective of the present study was to investigate the serum levels of IL-6, IL-8 and TNF- α and leptin in chronic renal failure patients submitted to haemodialysis.

In this study there were included two groups of subjects, one of them being chronic renal failure patients submitted to haemodialysis (n=60) and the other group was represented by healthy volunteers (n=30). Serum levels of inflammatory cytokines (IL-6, IL-8, TNF- α) and leptin were assessed before and after haemodialysis.

Leptin levels (9.609 ± 6.775 ng/mL) were high in haemodialysis patients if compared to the control group (4.585 ± 1.302 ng/mL) and after dialysis the concentration has decreased (6.466 ± 4.008 ng/mL). The concentrations of TNF- α (25.280 ± 4.640 pg/mL), IL-6 (8.073 ± 4.608 pg/mL) and IL-8 (34.370 ± 9.104 pg/mL) were high in patients submitted to haemodialysis in comparison with the group of healthy volunteers TNF- α (6.877 ± 1.447 pg/mL), IL-6 (2.014 ± 0.046 pg/mL), IL-8 (18.090 ± 5.840 pg/mL).

Haemodialysis patients showed elevated levels of leptin, IL-6, IL-8 and TNF- α . After dialysis it was observed a significant decrease of leptin level ($p = 0.0091$), whereas IL-8 increased significantly ($p < 0.0001$).

Rezumat

Leptina controlează metabolismul, echilibrul energetic și de asemenea poate intensifica inflamația prin inducerea activării și proliferării celulelor mononucleare. Acest din urmă efect al său reprezintă stimularea proliferării citokinelor pro-inflamatorii precum: interleukina-6 (IL-6), factorul de necroză tumorală (TNF- α), interleukina-8 (IL-8).

Obiectivele studiului de față au fost de a determina nivelurile serice ale IL-6, IL-8, TNF- α și ale leptinei la pacienții cu insuficiență renală cronică care sunt supuși hemodializei.

În acest studiu au fost incluse două loturi de pacienți, unul fiind alcătuit din pacienți cu insuficiență renală cronică supuși hemodializei ($n=60$), iar cel de al doilea lot fiind alcătuit din voluntari sănătoși ($n=30$). Nivelurile serice a citokinelor inflamatorii (IL-6, IL-8, TNF- α), precum și ale leptinei au fost determinate înainte și după hemodializă.

Nivelurile leptinei au fost crescute la pacienții hemodializați ($9,609 \pm 6,775$ ng/mL) în comparație cu cele ale lotului martor ($4,585 \pm 1,302$ ng/mL). Concentrațiile TNF- α ($25,280 \pm 4,640$ pg/mL), IL-6 ($8,073 \pm 4,608$ pg/mL), IL-8 ($34,370 \pm 9,104$ pg/mL) au fost crescute la pacienții supuși hemodializei în comparație cu cele ale pacienților clinic sănătoși TNF- α ($6,877 \pm 1,447$ pg/mL), IL-6 ($2,014 \pm 0,046$ pg/mL), IL-8 ($18,090 \pm 5,840$ pg/mL).

La pacienții hemodializați s-au determinat niveluri crescute ale leptinei, IL-6, IL-8 și TNF- α . După dializă a fost observată o scădere semnificativă a nivelului leptinei ($p = 0,0091$), iar concentrația serică a IL-8 a crescut semnificativ ($p < 0,0001$).

Keywords: haemodialysis, IL-6, IL-8, leptin, TNF- α .

Introduction

Hyperhomocysteinemia, reactive oxygen species together with hypoalbuminemia, malnutrition and atherosclerosis, the peroxisome proliferators-activated receptor, leptin and cytokines are the modulatory factors of inflammation [4, 25, 29]. The number of patients with end-stage renal disease increased worldwide [6]. In patients with chronic kidney disease and end-stage kidney disease, inflammation, mediated by pro-inflammatory cytokines, contributes to morbidity and mortality [14-27]. In our previous studies we showed that the patients submitted to haemodialysis had a high level of homocysteine associated with oxidative stress [1, 2].

They are potential factors involved in the initiation of inflammation, atherosclerosis and cardiovascular diseases in haemodialysis patients.

White adipose tissue is not only a reserve organ. It is also able to secrete or release hormones, peptides, and cytokines [16]. Leptin has a variety of physiological roles, such as control of metabolism, of energy balance by inhibiting energy intake and of intensifying the inflammatory process by mononuclear cell activation and proliferation [9, 22]. There is a decreased leptin serum level during fasting, period in which the reduction of energy expenditure appears, but the necessary energy to maintain the vital functions is assured. In this condition the low leptin serum concentration may lead to an immune suppression [1, 19].

The proinflammatory cytokines produced under the influence of leptin are IL-6, TNF- α , IL-8 [20].

The aim of our study was to assess the serum levels of IL-6, IL-8 and TNF- α and leptin in chronic renal failure patients submitted to haemodialysis before and after dialysis.

Materials and Methods

The study was performed on 60 chronic patients (29 females and 31 males) with renal failure submitted to haemodialysis in the Renamed Center of Haemodialysis from Oradea, Romania (median age 60, range 25-81 years) and 30 healthy controls (median age 50, range 30-70 years) (Table 1). The control group (CTRL) consisted of 15 healthy women and 15 healthy men. The renal chronic disease patients were treated by conventional haemodialysis, three times per week, each treatment lasting 3 to 5 hours. In the study there were included only functionally anephric patients, defined as having a urine output of 250 mL per day. All patients were medically stable at the time of selection, and for at least 3 months no modifications were performed on their form of therapy or dialysis prescription. During the study patients had no symptoms of infection. They did not receive drugs that would affect immune functions or blood transfusion. The exclusion criteria were patients younger than 18 years old, with an organ transplant within the previous year, acute obstructive uropathy or mentally disabled. They were dialyzed using high-flux polysulphone membrane dialyzers. Blood flow varied between 250-400 mL/min. Kt/V average was 1.58. The average haemodialysis (HD) therapy duration is 61 months, (11-240 months). The blood samples were collected before and immediately after the second haemodialysis session of the week, according to the guidelines for haemodialysis adequacy from arteriovenous fistula using Venosafe[®] Serum Gel + Clot activator Tubes (Terumo Europe) [12]. Specimens were transported to the laboratory immediately after collection and centrifuged at 1500 g for 10 min to separate serum. Written informed consent was obtained from all participants, prior to enrolment and the study was approved by the institutional ethical committee.

For measuring the concentration of IL-6, IL-8 and TNF- α we used an automated immunochemiluminescence assay (Immulite 1000 instrument, Siemens, Germany). Serum leptin level was determined using the DRG Leptin ELISA Kit—a solid phase enzyme-linked immunosorbent assay based on the sandwich principle on automated ELISA Adaltis instrument (Italy). Routine biochemical parameters on Advia 1800 instrument (Siemens, Germany) with Diasys reagents (Germany) were also performed. Urea was measured by urease – GLDH, enzymatic UV test (Cat. No.131019910917).

Statistical analysis

The Kolmogorov – Smirnov normality test was used to establish if the values of investigated parameters (leptin, TNF- α , IL-6, IL-8 and Urea) measured for each group (CTRL – control group, BEFORE – before haemodialysis and AFTER – after haemodialysis) had a normal distribution (Table III). Descriptive statistics of the data include both the normal distributed parameters (mean, variance, standard deviation, confidence intervals) and the non-normal distributed parameters (minimum, first quartile, median, third quartile and maximum), due the fact that within the data both normal (leptin, TNF- α , IL-8 and Urea) and non-normal (IL-6) distribution was present (Table III). Differences were tested with one-way analyse of variance: ANOVA test for normal distributed values and Kruskal-Wallis non-parametric test for non-normal distributed values. The *post hoc* tests with multiple comparisons were done with: Tukey's test (after parametric test) and Dunn's test (after non-parametric test). The values $p < 0.05$ were considered statistically significant. Differences between groups regarding urea levels BEFORE and AFTER haemodialysis were tested with the parametric t-test ($\alpha = 0.05$). Initial data for leptin, TNF- α , IL-6 and IL-8 presented large standard deviations. To address this issue it was used the ROUT ($Q = 10\%$) procedure to remove the outliers. After the outliers removal the number of data values may differ for each group of the studied parameters (Table III). Statistical analysis was done using GraphPad Prism version 5.00 for Windows (GraphPad Software Inc., San Diego California USA, www.graphpad.com). The reason for using a non-parametric one-way analysis of variance test is the normal and, respectively, non-normal distributed data of the groups.

Results and Discussion

The control (CTRL) group and the patients submitted to haemodialysis (BEFORE group) were subjected to anthropometric data analysis such as age, body mass index (BMI), and measurements of urea (Table I). The median age of the renal patients was 60 (range, 25-81 years) and CTRL group was 50 (range, 30-70 years). Data in both groups was normal distributed. The concentration of urea was higher in BEFORE group than in AFTER group (1.35 ± 0.35 g/L vs. 0.397 ± 0.149 g/L, respectively; $p < 0.0001$) (Table I and III).

Regarding the causes of the renal failure it can be mentioned that there were 2 patients with polycystic renal disease and one case of amyloidosis (Table II).

Table I
Clinical characteristics and biochemical parameters (urea), of CTRL and BEFORE haemodialysis groups

	CTRL group (n=30)	BEFORE group (n=60)	p
Female, N/total	15/30	29/60	
Male, N/total	15/30	31/60	
Age (years)	50 (30-70)	60 (25-81)	
BMI (kg/m²)*	23.16±1.26	26.31±5.68	0.026
Urea (g/L)	0.29 ± 0.09	1.35±0.35	0.0001
Duration of hemodialysis therapy (month)	-	61 (11-240)	
*BMI (Body Mass Index), the anthropometric parameter, represents the body weight in kilograms divided by their square height in meter.			

Table II
Etiology of chronic renal disease of haemodialysis patients

Etiology of hemodialysis patients	N
Tubulointerstitial nephropathy (TN)	17
Diabetic nephropathy (DN)	12
Chronic glomerulonephritis (CG)	25
Polycystic renal disease	2
Tubulointerstitial nephropathy (TN) + Diabetic nephropathy (DN)	3
Amyloidosis	1

After the outlier's removal of data regarding leptin, there were 43 patients in the group BEFORE, 44 patients in the group AFTER haemodialysis and 28 healthy controls (CTRL).

Table III
Descriptive statistics of Leptin, TNF- α , IL6, IL8, and Urea between groups

	LEPTIN			TNF- α			IL-6			IL-8			UREA		
	CTRL	BEFORE	AFTER	CTRL	BEFORE	AFTER	CTRL	BEFORE	AFTER	CTRL	BEFORE	AFTER	BEFORE	AFTER	
Number of values	28	43	44	30	59	59	21	50	54	48	30	54	60	60	
Minimum	2.124	0	0.3	3.7	13.7	11.4	2	2.03	1	16.1	7.33	13	0.65	0.1	
25% Percentile	3.571	4.77	3.105	5.75	21.6	20.7	2	4.935	2.67	27.75	13.88	32.98	1.1	0.29	
Median	4.314	9.12	5.955	7.1	23.7	26.4	2	6.785	5.455	33.55	17.45	63.75	1.335	0.38	
75% Percentile	5.155	14.79	9.69	7.95	28.5	29.7	2	9.685	8.923	41.88	21.9	87.53	1.595	0.4975	
Maximum	7.451	28.81	16.21	9.8	33.1	38.7	2.19	20.3	16.7	58.4	30	132	2.21	0.75	
95% CI of median															
Lower confidence	3.578	6.12	4.49	5.9	23.7	22.8	2	5.46	3.84	29.3	14.9	41.7	1.16	0.35	
Upper confidence	5.145	12.7	8.81	7.4	27.5	28.4	2	7.83	7.4	39.2	19.8	75.2	1.49	0.42	
Mean	4.585	9.609	6.466	6.877	25.28	25.110	2.014	8.073	6.064	34.370	18.090	65.250	1.347	0.397	
Std. Deviation	1.302	6.775	4.008	1.447	4.64	6.159	0.046	4.608	3.723	9.104	5.840	35.580	0.351	0.149	
Lower 95% CI of mean	4.080	7.524	5.247	6.336	24.070	23.510	1.993	6.763	5.048	31.730	15.910	55.540	1.256	0.359	
Upper 95% CI of mean	5.090	11.690	7.684	7.417	26.490	26.720	2.035	9.382	7.080	37.010	20.270	74.960	1.437	0.436	
Kolmogorov-Smirnov normality test															
KS distance	0.1238	0.1037	0.1147	0.0946	0.0677	0.1040	0.5231	0.2010	0.0969	0.0824	0.0935	0.1163	0.0691	0.1131	
P value	0.200	0.200	0.150	0.200	0.200	0.110	<0.0001	<0.0001	0.200	0.200	0.200	0.066	0.200	0.054	
Skewness	0.5253	0.3827	0.1860	-0.0310	-0.1027	-0.2466	3.4680	1.1820	0.6102	0.2748	0.3401	0.4091	0.1416	0.429	
Kurtosis	-0.0980	0.1335	-0.7567	-0.4307	-0.4135	-0.5320	12.0400	0.6893	-0.0431	-0.3705	-0.1412	-0.9714	-0.4604	-0.2026	

Compared with the control group (CTRL_Leptin, 4.585 ± 1.302 ng/L), leptin levels were significantly higher in patients on haemodialysis ($p < 0.0001$, for BEFORE_Leptin group and $p = 0.252$, for AFTER_Leptin group).

Compared with the group before haemodialysis (9.609 ± 6.775 ng/L) leptin level was lower ($p = 0.009$) in group after haemodialysis (6.466 ± 4.008 ng/L) (Table IV).

The concentration of cytokines was determined in all studied groups and the results are shown in Table IV - VI. Concentrations of serum TNF- α in renal disease patients (AFTER group) were significantly higher in comparison with CTRL group (25.280 ± 4.64 pg/mL vs. 6.877 ± 1.447 pg/mL, $p < 0.0001$) (Table IV). Our results shown that after the dialysis session, the concentration of TNF- α has not significantly changed (25.280 ± 4.64 pg/mL vs. 25.110 ± 6.159 pg/mL, respectively; $p = 0.9826$) (Table VI).

IL-6 serum concentrations were significantly higher in BEFORE group compared to CTRL group (8.073 ± 4.608 pg/mL *versus* 2.014 ± 0.046 pg/mL respectively; $p < 0.0001$). Haemodialysis session did not significantly change ($p = 0.1065$), the serum levels of IL-6 in patients (Table VI).

The levels of serum IL-8 in CTRL group was 18.090 ± 5.840 pg/mL and in BEFORE group, the levels of IL-8 were 34.370 ± 9.104 pg/mL ($p = 0.0102$) (Table IV). After haemodialysis session, a significant difference between serum IL-8 levels from BEFORE and AFTER groups was observed ($p < 0.0001$) (Table VI).

Table IV

Parameters (leptin and cytokines) values of the CTRL and BEFORE haemodialysis group

Parameters	CTRL	BEFORE	p
Leptin (ng/mL)¹	4.585 ± 1.302	9.609 ± 6.775	<0.0001
TNF -α (pg/mL)¹	6.877 ± 1.447	25.280 ± 4.640	<0.0001
IL-6 (pg/mL)²	2.014 ± 0.046	8.073 ± 4.608	<0.0001
IL-8 (pg/mL)¹	18.090 ± 5.840	34.370 ± 9.104	0.0102

The values are expressed as mean $\pm$ SD; the statistical test used was one-way analyse of variance ($\alpha = 0.05$): ANOVA¹ and Kruskal-Wallis²; with Tukey's¹ and Dunn's², respectively, *post hoc* tests for multiple comparisons.

Table V
Parameters (leptin and cytokines) values of the CTRL and AFTER
haemodialysis groups

Parameters	CTRL	AFTER	p
Leptin (ng/mL)¹	4.585 ± 1.302	6.466 ± 4.008	0.252
TNF -α (pg/mL)¹	6.877 ± 1.447	25.110 ± 6.159	< 0.0001
IL-6 (pg/mL)²	2.014 ± 0.046	6.064 ± 3.723	< 0.0001
IL-8 (pg/mL)¹	18.090 ± 5.840	65.250 ± 35.580	< 0.0001

The values are expressed as mean ± SD; the statistical test used was one-way analyse of variance (alpha = 0.05): ANOVA¹ and Kruskal-Wallis²; with Tukey's¹ and Dunn's², respectively, *post hoc* tests for multiple comparisons.

Table VI
Parameters (leptin and cytokines) values of groups BEFORE and AFTER
haemodialysis

Parameters	BEFORE	AFTER	p
Leptin (ng/mL)¹	9.609 ± 6.775	6.466 ± 4.008	0.0091
TNF -α (pg/mL)¹	25.280 ± 4.640	25.110 ± 6.159	0.9826
IL-6 (pg/mL)²	8.073 ± 4.608	6.064 ± 3.723	0.1065
IL-8 (pg/mL)¹	34.370 ± 9.104	65.250 ± 35.580	< 0.0001

The values are expressed as mean ± SD; the statistical test used was one-way analyse of variance (alpha = 0.05): ANOVA¹ and Kruskal-Wallis²; with Tukey's¹ and Dunn's², respectively, *post hoc* tests for multiple comparisons.

Data in literature demonstrate that there is an increased serum level of the pro-inflammatory cytokines such as IL-6, IL-8, IL-2 and TNF-α before and during haemodialysis and other studies indicated a moderate serum level [9-26, 28].

Our renal disease patients showed an increased serum TNF-α compared with the control group. After haemodialysis these values were unchanged. IL-6 activates acute phase proteins and suppresses albumin synthesis, enhances proliferation of B and T lymphocytes [13, 28]. We found no significant difference in IL-6, TNF-α level before and after haemodialysis in dialysis patients. IL-8 levels were significantly increased in patients after haemodialysis. It is released mainly by stimulated macrophages and fibroblasts [9]. This cytokine is also produced by human adipocytes in response to inflammatory stimuli such as IL-1, or TNF-α [17].

There are studies which underline that IL-8 represents an important predictor factor of cardiovascular mortality in haemodialysis patients [15, 24].

Kimmel PL et al. pointed out that serum pro-inflammatory cytokines levels such as IL-1, TNF- α , IL-6, IL-13 are significantly associated with an increased risk for mortality in patients submitted to haemodialysis [16]. IL-6 and TNF- α promote in liver the synthesis of the acute phase reactant, C-reactive protein, a cardiovascular risk factor [33].

Stenvinkel P et al. [30] suggested that IL-6 level correlates with the severity of atherosclerosis. The results of our paper are similar with other studies [5-18, 30].

Goldstein et al. (2006) demonstrated that TNF- α and IL-8 concentrations are significantly reduced by daily low-dose aspirin treatment [11]. The serum level of the pro-inflammatory cytokines IL-6, IL-8 and TNF- α in haemodialysis patients were found to remain suppressed 1 month after aspirin treatment was disrupted [11]. In haemodialysis the increase of inflammatory mediators are due to the activation of macrophages and neutrophils [10].

Leptin receptors have been found in neutrophils, monocytes, and lymphocytes [9]. In the end-stage of a renal disease a high concentration of leptin is associated with an increase in plasma IL-6, TNF- α and inflammatory markers [3, 18].

Conclusions

In conclusion, the results of our paper demonstrate that haemodialysis session significantly decreases leptin concentration and increase the level of IL-8. The proinflammatory state is maintained by the proinflammatory cytokines and leptin. The correlation between high serum leptin levels and clinical outcomes in end-stage renal disease has not been fully defined. The excess mortality has been attributed to cardiovascular disease and infection in chronic kidney disease. Further studies are needed to be carried out to prove the efficacy of treatments (natural extracts and drugs) in order to decrease inflammatory cytokines and leptin concentrations in haemodialysis patients so as to improve their prognostic.

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