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Relationship between leptin and chronic inflammatory state in uremic patients

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Abstract. – OBJECTIVE: To explore the relation between high leptin and inflammation in uremic patients.

PATIENTS AND METHODS: A group of 73 uremic patients in dialysis center of our Department were assigned as uremic group; a group of 30 healthy persons who were examined over the same period were regarded as control group. The level of body mass index (BMI), serum creatinine (SCr), blood urea nitrogen (BUN), leptin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-10, and the neutrophils phagocytosis function were compared in two groups.

RESULTS: BMI and IL-10 of uremic group were lower than the control group. The levels of SCr, BUN, leptin, TNF-α, IL-6 of uremic group were higher than the control group ($p < 0.05$). The neutrophils phagocytosis function in uremic group significantly decreases, compared to control group ($p < 0.05$). Using one-way ANOVA analysis, serum leptin was positively correlated with the level of TNF-α, IL-6 ($r = 0.58$, 1.00 respectively, $p < 0.05$), and was negatively correlated with the level of IL-10 ($r = –0.45$, $p < 0.05$).

CONCLUSIONS: The high level of leptin and correlated inflammation were involved in the initiation and development of uremia; moreover, leptin was an important mediator.

Key Words:
Uremia, Leptin, Interleukin, Inflammation.

Introduction

Leptin had the main functions of suppressing appetite, increasing energy consumption and inhibiting adipose synthesis. In addition, leptin played a role in regulating the inflammation and immune of the whole body and participating in a variety of pathological processes of chronic renal failure. The retrospective studies of patients with uremia would contribute to analyze the possible causative factors and provide more experimental data for prevention and treatment.

Patients and Methods

Patients and Groups

Seventy-three uremic patients in our Urology Dialysis Centers were observed, including 26 males and 47 females. The patients were between 20 to 78 years old, and the average age was 47.3 ± 15.1. Among these patients, there were 27 cases with chronic nephritis, 15 cases with hypertensive nephropathy, 12 cases with diabetic nephropathy, 6 cases with lupus nephritis, 4 cases with polycystic kidney, and 9 cases with IgA nephropathy. All patients were in stable condition, had no obvious infection, without trauma, surgery, cancer, connective tissue disease, or liver disease within the past month. The observed cases were not organ transplant patients or pregnant women. These patients had no history of blood transfusion, did not use hormones or immunosuppressant. There was no cardiovascular event occurred in these selected patients within the past month. Thirty healthy persons who took medical examinations in our medical examination center were observed as control group, including 11 males and 19 females. The subjects in control group were between 20 and 50 years old, and the average age was 45.5 ± 13.3. Body mass index (BMI) was matched. The outcome measures are described in Table I.

Sample Collection, Separation, and Preservation

Control group and non-dialysis patients were advised to take a 5-ml fasting venous blood in the second morning after hospitalization. Hemodialysis patients were advised to take fasting venous blood in the morning of the dialysis day. After 30-min standing, the collected blood samples were centrifuged at 3000 rpm for 10 min. The separated serum samples were stored in –70°C. After all collections were done, the samples were detected.

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Table I. Comparison of outcome measures of two groups ($x \pm s$).

Group	Case	BMI (kg/m²)	SCr (μmol/l)	BUN (mmol/l)	Phagocytic rate (%)	Leptin (ng/l)	TNF-α (ng/l)	IL-6 (ng/l)	IL-10 (ng/l)
Uremic group	73	25.31 ± 2.68[a]	643.37 ± 21.94[a]	75.03 ± 21.94[a]	10.73 ± 1.66[a]	31.86 ± 5.43[a]	88.80 ± 7.87[a]	65.04 ± 8.59[a]	16.94 ± 5.28[a]
Normal group	30	15.19 ± 1.01	75.03 ± 19.19	5.21 ± 1.93	29.41 ± 2.63	14.57 ± 2.55	57.23 ± 18.15	39.14 ± 8.61	19.67 ± 10.83

Note: Compared to control group, [a]$p < 0.05$.

Outcome Measures

Height and weight of the two groups were measured in blood collection day. BMI was calculated (BMI = Weight/height). Serum creatinine (SCr) was measured by oxidase method. Blood urea nitrogen (BUN) was measured by urease method. The concentration of serum leptin was measured by radioimmunoassay, and the measuring kit was brought from DLS Company Chicago, IL, USA. Content of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) in serum was measured by enzyme-linked immunosorbent assay. TNF-α, IL-6, and IL-10 kits were imported sub-packages of R&D. According to instructions of the kits, double-blank-control was used. It was measured by enzyme-labeled instrument (TECAN Company, Männedorf, Switzerland) at the wavelength of 450 nm.

Phagocytic function of neutrophils in peripheral blood was measured as follows: yeasts were cultured in 37 for 24 h, washed twice by balanced salt solution, counted, taken Giemsa staining after smearing, and then the activity was measured. A quantity of 2.5-ml blood was drawn and put into a sterile heparin anticoagulant tube to count leukocytes and neutrophils. Then 0.2-ml yeast-balanced salt solution was added to 0.8-ml heparin anticoagulant whole blood (The ratio of yeast to neutrophil was 2:1, cultured in 37°C for 1 h). Naturally precipitated leukocytes were absorbed for smears and Giemsa staining. The total number of yeast swallowed by 100 neutrophils was counted. Viable cells were stained to blue, dead cells were non-colored, or not stained at center and (or) at both ends. The phagocytic percentage was counted by the ratio of neutrophils that swallowed yeast to 100 neutrophils.

Statistical Methods

The statistical analysis was taken by using SPSS18.0 statistical software (SPSS Inc., Chicago, IL, USA). The results were indicated by average ± standard deviation. Multiple samples were compared using variance analysis and LSD test, and correlations were analyzed by Pearson's correlation analysis. It would be statistically significant, when the difference was $p < 0.05$.

Results

BMI and IL-10 of uremic group were lower than the control group. The levels of SCr, BUN, leptin, TNF-α, and IL-6 of uremic group were higher than

the control group ($p < 0.05$). The neutrophils phagocytosis function in uremic group (10.23±1.16) significantly decreased compared to control group (29.41 ± 2.63, $p < 0.05$, Table I).

Using one-way ANOVA analysis, serum leptin of uremic group was positively correlated with the level of TNF-α, IL-6 ($r = 0.58$, 1.00 respectively, $p < 0.05$), and was negatively correlated with the level of IL-10 ($r = -0.45$, $p < 0.05$).

Discussions

Hyperleptinemia could cause malnutrition and participated in uremia concurrent cachexia. Especially for patients with obesity-associated nephropathy or type 2 diabetes, high levels of leptin promoted proliferation and sclerosis of glomerular, reduced insulin sensitivity, caused impaired glucose tolerance, promoted vascular calcification, and enhanced the risk of cardiovascular disease[1]. However, the reasons of high level of leptin in uremic patients were not very clear. Montecucco et al[2] thought that the main reasons of hyperleptinemia were decreased glomerular filtration rate in uremic patients and significantly reduced blood flow of kidney that caused decreased ability to renal clearance of leptin[3]. Recently, it was found by studies that the increased secretion of leptin in fat cells of uremic patients may be one of the reasons that caused hyperleptinemia. It prompted that main reasons of hyperleptinemia were increased secretion and/or decreased clearance. This study showed that the main performance of body micro-inflammation of uremic patients was the imbalance of pro-inflammatory cytokines and anti-inflammatory cytokines that accelerated atherosclerosis and aggravated malnutrition of uremia. Therefore, the micro-inflammation of whole body stimulated leptin production. The main mechanism might be by inhibiting p38 mitogen-activated protein kinase and Src kinase to reduce the chemotaxis of neutrophil[2].

Chronic micro-inflammation involved in occurrence and development of many pathological processes such as anemia, malnutrition, atherosclerosis that were the complications of uremia[4,5]. It was gradually discovered in recent years that abnormal immune function and metabolic disorders of the cytokines happened in uremic patients. The major cytokines were TNF-α IL-1, IL-6, IL-8, etc. TNF-α was a polypeptide produced by monocyte-macrophage cells. TNF-α participated in many physiological and immune processes and had the functions of anti-cancer and anti-infection. However, excessive production or release of TNF-α and imbalance between other cytokines could cause pathological changes such as fever and shock. This high pro-inflammatory cytokines hyperlipidemia could cause malnutrition, chronic inflammation, atherosclerosis in hemodialysis patients. TNF-α adjusted synthesis of CRF (chronic renal failure) in liver and induced expression of IL-6. High levels of TNF-α and IL-6 could cause glomerular mesangial matrix proliferation, mesangial cell proliferation, glomerulosclerosis, and deterioration of the disease progress in patients with CRF[6]. TNF-α brought platelet to accumulate in endothelial cells that led to thrombose. In addition, TNF-α induced mesangial cells to produce tissue factor-like active substances and increased the activity of procoagulant. That affected coagulation and fibrinolytic systems. This showed how to block TNF-α was the key to control of chronic inflammation. It was reported that IL-6 and TNF-α were produced by monocytes and lymphocytes that were stimulated by high level of leptin, and this process was achieved through the role of leptin receptor[7]. In the other side, cytokines could also promote the secretion of leptin. The relationship between leptin and cytokine was synergistic stimulation. IL-10 could regulate the expression of pro-inflammatory cytokines such as IL-6[8]. It was shown in recent studies that low expression of IL-10 gene polymorphism in hemodialysis patients was closely related to morbidity and mortality rate of cardiovascular diseases. In this research, there was a noticeable difference in serum IL-10 level between uremic group and control group. Serum leptin was negatively correlated with the level of IL-10. It showed that high level of leptin stimulated the production of pro-inflammatory cytokines IL-6 and inhibited the expression of anti-inflammatory cytokine IL-10. That aggravated the inflammatory state of patients with uremia and there was closely positive correlation between hyperleptinemia and inflammation in patients with CRF. Overseas studies have shown, micro-inflammation state of uremia could cause increased secretion of leptin in fat cells. Among these inflammatory cytokines related with micro-inflammatory, TNF-α was a precursor of anorexia cytokine that regulates appetite and eating behavior, and IL-6 was an important inflammatory cytokines[9] that promoted the catabolism. Both of TNF-α and IL-6 could

inhibit synthesis of protein, promote muscle breakdown, and cause malnutrition.[7] These results indicated that there was a close relationship between hyperleptinemia and malnutrition with inflammation in patients with end-stage renal diseases. This study found that decline of granulocyte-consuming rate in uremia patients had a significant difference from control group. That prompted the uremia patients had leukocyte dysfunction. We initially thought that the mechanism of decline of granulocyte-consuming rate was that micro-inflammation caused by hyperleptinemia led to immune dysfunction. Therefore, uremia patients were more vulnerable to invasion of pathogenic microorganism. Studies show that atherosclerosis was an inflammatory response. There was a close relationship between chronic inflammatory state and cardiovascular disease in uremia patients[10]. Some researchers did 30 months follow-up of 438 patients with maintenance hemodialysis and found that serum leptin concentration and carotid artery intima-media thickness were significantly positively correlated. They also pointed out that leptin by regulating inflammatory response, promoting platelet aggregation and affecting clotting factors, and other pathways was involved in atherosclerosis[11].

Conclusions

This research showed that leptin could be used as a clinical indicator of the detection of chronic micro-inflammatory state. We will focus on leptin through which signal transduction, what kind of mechanism involved in the inflammatory response and the development.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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