

Features of Lipid Metabolism and Level of Pro-inflammatory Cytokines in Patients with Type-2 Diabetes and with Diabetic Nephropathy Depending on the Stage of Chronic Kidney Disease

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Abstract

Purpose: Study of the role and relationship of lipid metabolism parameters and level of pro-inflammatory cytokines in patients with type-2 diabetes with diabetic nephropathy (DN) depending on the stage of chronic kidney disease (CKD).

Materials and methods: 240 patients with type-2 diabetes were examined in the early stages of DN and CKD.

Results: The development of DN was accompanied by an increase in the level of pro-inflammatory cytokines and impaired lipid metabolism (hypertriglyceridemia) in patients with type 2 diabetes. A negative correlation was found between the level of triglycerides (TG) and the glomerular filtration rate (GFR) ($r=-0.43$) and a direct relationship between the level of IL-6 and TG ($r=0.48$).

Findings: An increase in the level of pro-inflammatory cytokines and TG increases the risk of the development and progression of DN and CKD.

Keywords: Type 2 diabetes; Diabetic nephropathy; Chronic kidney disease; Hypertriglyceridemia; Pro-inflammatory cytokines (IL-6, IL-8, TNF- α)

Introduction

Kidney damage in diabetes is a major problem in modern diabetology, since the development of diabetic nephropathy (DN) dramatically reduces the overall survival of diabetic patients and is one of the most common causes of end-stage renal failure. The number of patients with DN has a steady upward trend due to an increase in the frequency of diabetes itself, a decrease in the mortality of patients from acute complications of diabetes and an increase in the overall life expectancy of patients. In recent decades, there has been a rapid increase in the number of patients in need of renal replacement therapy in all countries of the world (dialysis, kidney transplantation).

The mechanisms for the development of chronic kidney disease (CKD) in patients with type 2 diabetes are complex and diverse. One of the key links in the development and progression of microangiopathic complications of diabetes is "diabetic dyslipidemia", the basis of which pathogenesis is enhanced mobilization of free fatty acids from peripheral adipocytes to the liver, where they serve as a substrate for the assembly of low density triglyceride saturated lipoproteins (LDL). At the same time, the activity of the enzyme lipoprotein lipase, which splits lipoproteins saturated with triglycerides (TG), decreases [1-7].

Hypertriglyceridemia plays a special role in the development of CKD, increasing the risk of its development in diabetes, namely, increasing the percentage of especially atherogenic small, dense LDL

and reducing the concentration of high-density lipoproteins (HDL). Thus, not only quantitative, but also qualitative changes in the parameters of lipid metabolism occur [8-19].

Currently, the most promising markers of impaired immunoregulation in inflammatory diseases include protein histohormones-cytokines [2-4]. Cytokine production is of an activation nature and provides information exchange between cells involved in the inflammatory process. A feature of the genesis of cytokinemia in diabetes is that in patients as the disease progresses, the number of cellular structures with high production of cytokines increases. The study of the role of cytokinemia and imbalance between pro-inflammatory and anti-inflammatory cytokines in the process of developing complications of diabetes will provide additional laboratory criteria for assessing the severity, nature of the course, predicting disease outcomes, will become the basis for finding new methods, more effective schemes and treatment approaches [7].

Studies of the last 10 years show that inflammation (especially pro-inflammatory cytokines) is predictor of the development of vascular diabetic complications, namely DN [14]. The high level of pro-inflammatory cytokines in most patients with diabetes complications confirms their participation in the progression of diabetic angiopathies [9-12,16-18].

It has been established that the development of vascular complications in diabetes is associated with a chronic immunoinflammatory process and with the formation of immune complexes [13]. Activated monocytes-macrophages, immunoglobulins, cytokines, adhesion molecules, products of final glycosylation are directly involved in this. Pro-inflammatory cytokines (IL-6, TNF- α , etc.) have

been shown to stimulate the initiation and progression of vascular complications in diabetes [13-20].

Objective

To study the role and relationship of lipid metabolism and level of pro-inflammatory cytokines in patients with type-2 diabetes with DN, depending on the stage of CKD.

Material and Methods

In this work 320 people were examined, of which 240 were patients with type 2 diabetes and 80 were patients without carbohydrate metabolism disorders. The average age of patients was 54.51 ± 1.06 years; the duration of the disease was 2.05 ± 0.21 years. Patients with diabetes were in a state of compensation of carbohydrate metabolism (the average level of glycated hemoglobin (HbA1c) is $6.25 \pm 0.15\%$). The average body mass index (BMI) is $30.29 \pm 0.63 \text{ kg/m}^2$ (obesity of the 1st stage) [6]. Arterial hypertension (AH) 1 tbsp. registered in 90% of cases [10].

Four groups of patients (matched by sex, age and degree of obesity) were formed for the study: Group 1-control group-80 people; Group-2 patients with diabetes with normoalbuminuria (NAU)-80 people. Patients with microalbuminuria (MAU) were divided by creatinine level and glomerular filtration rate (GFR) into groups: Group 3-patients with MAU, chronic kidney disease (CKD) stage 1-80 people, group 4 - patients with MAU, CKD stage 2-80 people [5]. The average level of NAU in patients of group 2 was $10.77 \pm 0.54 \text{ mg/l}$. The average level of UIA in groups 3 and 4 was $50.35 \pm 2.62 \text{ mg/l}$.

Clinical and biochemical examination included determining the level of UIA by the method of borate affinity analysis (analyzer-reflectometer "NICOcard Reader-II"); the creatinine index by the Yaffe kinetic method (automatic biochemical analyzer "SAPPHIRE 400"). GFR was calculated using the MDRD formula.

Serum total cholesterol (OH) and TG were determined on an automatic biochemical analyzer "SAPPHIRE 400". The measurement of

HDL-C was carried out on the basis of the enzymatic method after precipitating the precipitates of LDL and VLDL formed under the influence of precipitants (magnesium, phosphotungstic acid). Then, based on the determination of OH, TG and HDL at the Friedwald W.T. (1972) estimated the concentration of LDL: $\text{LDL (mmol/l)} = \text{total cholesterol} - \text{HDL} - (\text{TG (mmol/l)} / 2.2)$.

The level of pro-inflammatory cytokines (interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF- α) in the serum was assessed by a chemiluminescent method on an immunoassay analyzer (Immulite 1000, USA).

Statistical processing of the results was performed using the Statistica 6.0 statistical software package (StatSoft.Ins, USA) and Microsoft Excel. The results obtained were subjected to variation analysis with the calculation of the arithmetic average and its errors for each group. To determine the degree of interrelation between the studied parameters, the Spearman rank correlation coefficient was calculated. The threshold level of statistical significance was taken as $p < 0.05$.

Results and Discussion

When analyzing the blood lipid spectrum, a significant increase in the TG level was found in patients with type 2 diabetes with CKD-2 (group 4) compared with the group of patients with type 2 diabetes with NAU (group 2) ($1.95 \pm 0.22 \text{ mmol/l}$ and $1.39 \pm 0.11 \text{ mmol/l}$, respectively ($p < 0.0001$)). At the same time, the level of OH and LDL is slightly changed and remains within the normal values in all the studied groups Table 1.

Hypertriglyceridemia determines the progression of DN, as evidenced by negative correlation ($r = -0.43$). In this regard, the most reliable it is advisable to determine the level of TG, even with normal or slightly elevated levels of TC and LDL. This fact is confirmed in the works of several other authors [11-13].

| Analyzed indicator | Group 1 control (n=80) | Group 2 NAU (n=80) | MAU | |
|--|------------------------------|--------------------------|---|---|
| | | | Group 3 CKD-1 (n=80) | Group 4 CKD-2 (n=80) |
| Cholesterol, mmol/l | 4.83 ± 0.10 | $5.53 \pm 0.15^{***}$ | $5.81 \pm 0.12^{***, \wedge \wedge}$ | $5.73 \pm 0.13^{***, \wedge}$ |
| LDL, mmol/l | 1.62 ± 0.11 | $1.29 \pm 0.22^*$ | $1.40 \pm 0.06^{**}$ | $1.26 \pm 0.05^{***, \# \#}$ |
| Tg, mmol/l | 1.00 ± 0.08 | $1.39 \pm 0.11^{***}$ | $1.77 \pm 0.10^{***, \wedge \wedge \wedge}$ | $1.95 \pm 0.22^{***, \wedge \wedge \wedge}$ |
| HDL, mmol/l | 2.89 ± 0.11 | $3.26 \pm 0.15^{***}$ | $3.44 \pm 0.13^{***}$ | $3.22 \pm 0.13^{***, \#}$ |
| Differences in comparison with group 1: * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$; in comparison with group 2: \wedge $p < 0.05$; $\wedge \wedge$ $p < 0.01$; $\wedge \wedge \wedge$ $p < 0.0001$; in comparison with group 3: $\#$ $p < 0.05$; $\# \#$ $p < 0.0001$. | | | | |

Table 1: Indicators of lipid metabolism in patients with type 2 diabetes

Thus, the level of TG is prognostically significant in the development of DN and CKD.

Along with hypertriglyceridemia in the examined groups, as CKD develops, the level of another risk factor for vascular complications, the level of IL-6, increases. The confirmation of the obtained results is a positive correlation between the level of TG and IL-6 ($r = 0.48$).

As we can be seen from Table 2 the content of IL-6 increases with the progression of CKD. In patients with MAU in the 4th group, this indicator was significantly higher than in the control group ($3.71 \pm 1.25 \text{ pg/ml}$ and $2.30 \pm 0.08 \text{ pg/ml}$, respectively ($p < 0.001$)).

| Analyzed indicator | Group 1 control (n=80) | Group 2 NAU (n=80) | MAU | |
|--------------------|------------------------------|--------------------------|----------------------------|----------------------------|
| | | | Group 3 CKD-1 (n=80) | Group 4 CKD-2 (n=80) |
| IL-6, PG/ml | 2.30 ± 0.08 | 2.91 ± 0.62 | 3.51 ± 1.25 | 3.71 ± 0.89* |
| IL-8, PG/ml | 8.98 ± 0.32 | 11.44 ± 0.80** | 13.40 ± 1.26**.^ | 11.52 ± 1.26**.# |
| TNF-α, PG/ml | 6.67 ± 0.43 | 11.28 ± 0.93** | 12.13 ± 1.25** | 13.48 ± 0.93**.^ |

Differences in comparison with group 1: *p<0.001; **p<0.0001; in comparison with group 2: ^p<0.01; .p<0.001; in comparison with group 3: #p<0.05.

Table 2: Indicators of the cytokine spectrum in patients with type 2 diabetes.

In type 2 diabetes, numerous disorders are found that are characteristic of the acute phase of the body's response, initiated by cytokines IL-6 and TNF-α; these processes are most pronounced in the presence of NAM. It is known that TNF-α performs regulatory and effector functions in the immune response and inflammation, is an inducer of IL-8, which in turn activates neutrophils, causes their chemotaxis to the focus of inflammation [13]. Elevated levels of IL-8 are associated with chronic and acute inflammatory conditions and correlate with tissue infiltration of neutrophils [13].

The results of our study (Table 2) show that the content of IL-8 and TNF-α significantly increased compared with the control group and is 11.52 ± 1.26 pg/ml and 13.48 ± 0.93 pg/ml, respectively, which indicates the presence of a chronic inflammatory process in patients with CKD ($p<0.0001$). The obtained data are confirmed by the presence of a positive correlation relationship between these indicators ($r=0.50$).

According to Nefed [11] 3 stages of the development of the immune response of the organism in patients with type 2 diabetes with microangiopathies were highlighted: Stage 1 — increase in the content of pro-inflammatory cytokines; Stage 2 - cytokinemia; Stage 3 - reduction of cytokine-producing ability of cells of the immune system [11].

Analyzing the data in Table 2, a decrease in the cytokine-producing activity of mononuclear cells, in particular IL-8, is observed, which may indicate the beginning of the development of immunosuppression in patients with type 2 diabetes with CKD-2.

The quantitative content of key pro-inflammatory cytokines (IL-6, IL-8, TNF-α) and the ability of blood mononuclear cells to produce them reflect the severity of vascular complications, which is confirmed in our study by the presence of a correlation between the level of IL-8 and GFR ($r=-0.57$) and the level of TNF-α and GFR ($r=-0.43$) [11].

Thus, there is no doubt that the activation of indicators of pro-inflammatory cytokines plays a significant role in the development of CKD in patients with type 2 diabetes. An increase in the level of IL-6 at the stage of MAU characterizes an imbalance of the cytokine regulation of the immune response in patients with type 2 diabetes, and a decrease in the level of IL-8 suggests a decrease in the immune response in patients with type-2 diabetes with CKD-2.

Findings

1. Evaluation of lipid metabolism allowed determining the role of hypertriglyceridemia in the progression of DN. An increase in triglycerides aggravates the course of DN, which is confirmed by a negative correlation between the level of TG and GFR ($r=-0.43$).

2. The development and progression of DN is accompanied by an increase in the level of pro-inflammatory cytokines (IL-6, IL-8, TNF-α) and the presence of a correlation between the level of IL-8 and GFR ($r=-0.57$) and TNF-α and GFR ($r=-0.43$) in patients with type-2 diabetes.

3. The level of pro-inflammatory cytokines increases simultaneously with changes in blood lipid metabolism in patients with type 2 diabetes—positive correlation between the level of TG and IL-6 ($r=0.48$).

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