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PRO-INFLAMMATORY CYTOKINES IN PATIENTS WITH CHRONIC KIDNEY DISEASE: INTERLEUKIN-6 IN FOCUS

Abstract

Objective of the study. To assess the clinical and pathogenetic significance of serum interleukin-6 (IL-6) in patients with chronic kidney disease. Materials and methods. A cross-sectional study enrolled 288 patients with chronic kidney disease (CKD) aged 16 to 86 years, average age (54.5 ± 14.5) years. The study enrolled 146 (50.7%) women and 142 (49.3%) men. Depending on the value of estimated glomerular filtration rate (eGFR), all the examined patients were divided into two groups: 1st (n = 154) — persons with eGFR > 60 ml/min; 2nd (n = 134) — patients with eGFR < 60 ml/min, i.e. renal failure. CKD was identified when there was evidence of damaged and/or reduced renal function. Glomerular filtration rate was calculated using the Hoek equation based on measurement of serum cystatin C, and severity of CKD was based on eGFR values. All patients had concentration of creatinine, cystatin C and IL-6 in their blood serum studied.

Results. In the 2nd group of patients with eGFR below 60 ml/min, average age [(57.9 ± 14.5) years vs. (51.6 ± 13.9) years; p < 0.05], systolic blood pressure [(142 ± 24) mm Hg vs. (133 ± 22) mm Hg; p < 0.05], cystatin C [1.815 (1.430–3.070) mg/l vs. 0.980 (0.900–1.100) mg/l; p < 0.05] and IL-6 [2.761 (1.400–6.495) pg/ml vs. 1.754 (0.849–3.226) pg/ml; p < 0.05] levels in blood serum were significantly higher compared with the 1st group. An inverse correlation was found between serum IL-6 and eGFR level (r = −0.144; p = 0.018).

Conclusion. In patients with chronic kidney disease with an eGFR level below 60 ml/min, an increase in systolic blood pressure and serum IL-6 concentration was observed. In chronic kidney disease, an increase in the content of IL-6 was accompanied by a decrease in glomerular filtration rate and an increase in diastolic blood pressure.

Key words: chronic kidney disease, glomerular filtration rate, interleukin-6, inflammation, progression, cardiovascular complications

Conflict of interests
The authors declare no conflict of interests.

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Introduction

The problem of early diagnosis, prevention and treatment of chronic kidney disease (CKD) in the clinical course of adult diseases remains relevant for modern-day medicine. Timely assessment of the severity of CKD and associated cardiovascular and cerebral complications is of great practical importance for primary and secondary prevention [1]. All researchers recognize the multifactorial nature of the origin and progression of CKD [2, 3]. The widespread prevalence of diseases accompanied by CKD and cardiovascular events necessitates further study of this problem [4]. A number of studies established that in the occurrence and progression of CKD, a change in the interleukin (IL)-6 level in serum [5, 6] and kidney tissue [7, 8] is of importance. IL-6 is absent in a healthy kidney, and its plasma level ranges from 1 to 2 pg/ml [8]. According to some researchers, IL-6 is produced by activated monocytes/macrophages, fibroblasts, endothelial cells, as well as mesangial and epithelial cells of the renal tubules [9, 10]. According to current data, IL-6 is one of the most important mediators of the acute inflammation phase [11–13]. In acute inflammatory diseases, IL-6 secretion is maximally stimulated, and its plasma level can reach values of 1,000 pg/ml. In muscles and adipose tissue, IL-6 stimulates the mobilization of energy, which leads to an increase in body temperature, and in the liver, it is the main stimulator of the synthesis of acute phase proteins. In addition, this cytokine stimulates the proliferation and differentiation of B- and T-cells, as well as leukocytepoiesis. On the cell surface, IL-6 binds to a heterodimeric receptor complex, which is referred to as the type I cytokine receptor and consists of two transmembrane proteins: IL-6 receptor and gp150 (CD130). This receptor binds several other interleukins, which belong to the IL-6 superfamily based on this feature. The role of pro-inflammatory cytokines in the development of cardiovascular complications is being actively studied. However, the relationship between serum IL-6 and CKD markers remains poorly understood.

Study objective

To evaluate the clinical and pathogenetic significance of serum IL-6 in patients with chronic kidney disease.

Materials and methods

We examined 288 patients with chronic kidney disease (CKD) aged 16 to 86 years, the average age was (54.5 ± 14.5) years. The study involved 146 (50.7%) women, average age (54.5 ± 14.6) years, and 142 (49.3%) men, average age (54.6 ± 14.4) years (p > 0.05). Depending on the estimated glomerular filtration rate (eGFR), patients were divided into two groups: the 1st (n = 154) — individuals with eGFR > 60 ml/min (88 women / 66 men) and the 2nd (n = 134) — patients with eGFR < 60 ml/min, i.e. with kidney disease (58 women / 76 men). According to the international guidelines, CKD was diagnosed according to signs of damage and/or decreased renal function [14]. To assess the severity of CKD, eGFR was used; its value was calculated using the Hoek equation proposed in 2003: GFR = 80.35 / Cystatin C — 4.32 [15]. The inclusion criterion was the presence of CKD signs. The study did not enroll patients on corticosteroid and immunosuppressive therapy, with thyroid disease, fever and CKD stage 5D. In all patients, height (cm) and body weight (kg) were measured, body mass index (BMI) was determined by the Kettle method: BMI, kg/m² = weight, kg / height, m². Systolic and diastolic blood pressure (SBP and DBP, mm Hg), heart rate (HR, beats/min) were measured by
conventional methods. Serum concentrations of cystatin C (mg/l) and creatinine (μmol/l) were analyzed in all patients. The serum IL-6 level (pg/ml) was determined using the Vector-Best JSC (Novosibirsk, Russia) reagent kit via enzyme-linked immunosorbent assay. Study results were registered on the ChroMate Microplate Reader (USA, 2015). According to the kit manufacturer’s instructions, a concentration of 10 pg/ml was taken as the upper limit of the standard.

Statistical analysis was carried out using the Statistica 10.0 software package (Statsoft, USA), which enables to perform parametric and nonparametric analysis. The results are presented as arithmetic mean (M) and standard deviation (SD) for characters with a normal distribution, and interquartile range (25th quartile; 75th quartile) in case of a nonparametric distribution of the characteristic. Intergroup comparisons were carried out using the nonparametric Mann — Whitney test, and in the presence of signs with a normal distribution, the Student t-test. The linear relationship between the variables was measured using the Pearson correlation test. The null hypothesis of the absence of differences and relationships was rejected at p < 0.05.

Results

In the patients examined, the cause of CKD was: overweight and obesity — 206 (71.5%), symptomatic hypertension — 48 (16.65%), essential hypertension — 88 (30.5%), type 2 diabetes mellitus (DM 2) — 62 (21.5%), chronic obstructive pulmonary disease — 58 (13.1%), chronic pyelonephritis — 23 (7.9%), chronic glomerulonephritis (CGN) — 42 (14.5%), stable forms of coronary heart disease — 66 (22.9%) and gout — 2 (0.6%). The number of patients with primary kidney disease in the analyzed subgroups did not significantly differ. The proportion of patients with chronic pyelonephritis was 15 (56.5%) in the 1st and 10 (43.5%) in the 2nd group. CGN was present in 18 (42.8%) patients in the 1st and 24 (57.2%) in the 2nd group (p > 0.05). More than half of the examined patients [154 (53.4%)] had CKD stage 1 and 2. At the same time, the proportion of patients with severe nitrogen excretory dysfunction was insignificant (Table 1) and amounted to 46 (15.9%). There were significantly more patients with CKD stage 3A than with stage 3B, 20.5% and 10.1% respectively (p < 0.05); 7.2% of the examined patients had end-stage renal disease (Table 1).

The average age of the examined patients in the 2nd group was significantly higher (p < 0.05). The gender composition of the examined groups was not similar (in the 1st group, the proportion of females was higher compared to the 2nd group).

BMI, diastolic blood pressure and heart rate in the study groups were equivalent (Table 2). SBP was significantly higher in patients of the 2nd group compared with the 1st group ((142 ± 24) mm Hg versus (133 ± 22) mm Hg; p < 0.05). According to the distribution criteria, in patients of the 2nd group, serum cystatin C and creatinine were significantly higher (p < 0.05), and eGFR was significantly lower (p < 0.05). The median and interquartile serum IL-6 values were significantly higher in patients with eGFR < 60 ml/min, i.e. in the 2nd group [2.761 (1.400–6.495) pg/ml versus 1.754 (0.849–3.226) pg/ml; p < 0.05] than in the 1st group.

The conducted correlation analysis revealed the presence of a reliable negative relationship between serum IL-6 and eGFR value (r = −0.144; p = 0.018).

Table 1. The population of examined patients with CKD

<table>
<thead>
<tr>
<th>Stages of chronic kidney disease, NKF KDOQI, 2002</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1, kidney damage with normal or elevated GFR</td>
<td>46 (16.0%)</td>
</tr>
<tr>
<td>Stage 2, kidney damage with a mild GFR decrease</td>
<td>108 (37.5%)</td>
</tr>
<tr>
<td>Stage 3A, moderate GFR decrease</td>
<td>59 (20.5%)</td>
</tr>
<tr>
<td>Stage 3B, significant GFR decrease</td>
<td>29 (10.1%)</td>
</tr>
<tr>
<td>Stage 4, severe GFR decrease</td>
<td>25 (8.7%)</td>
</tr>
<tr>
<td>Stage 5, end-stage renal disease</td>
<td>21 (7.2%)</td>
</tr>
</tbody>
</table>

Notes. CKD — chronic kidney disease; NKF KDOQI — National Kidney Foundation Kidney Disease Outcomes Quality Initiative; GFR — glomerular filtration rate; n — the number of patients
In addition, there was a tendency for a close relationship between serum IL-6 and diastolic blood pressure ($r = 0.119; p = 0.050$).

**Discussion**

The practical significance of identifying groups of patients with CKD and pro-inflammatory cytokinemia is the need to assess not only cardiovascular, but also renal complications [12, 13]. The damaging effects of pro-inflammatory cytokines on the kidney were first described by R. J. Shalhoub et al. in 1974 [16]. After 20 years, C. Luttricken et al. (1994) described the molecular mechanism of IL-6 action [17]. It was shown that with a change in the structure of the glomeruli, interstitium, and perivascular zone, cytokine receptors can be expressed, thereby triggering inflammation and further progression of pathological changes [18]. In particular, the expression of IL-6 receptors on mesangial cells indicates the activation of the immune process in the tissue, i.e. that mesangial cells began to ingest IL-6, while losing their ability to ingest immune complexes [7]. The role of IL-6 in proliferative and inflammatory processes in the kidney tissue is well demonstrated in the work of I. A. Rakityanskaya et al. (1998) [7]. Numerous cross-sectional studies have also shown that IL-6 secretion moderately increases in chronic mild inflammatory process, which is typical of CKD [11–13]. This fact is clearly reflected in our study, where (Table 2) in patients with renal failure, a statistically significant IL-6 increase was noted. We also managed to demonstrate, although not a strong, but reliable relationship between eGFR value and serum IL-6 (Table 3). Earlier, we showed a close relationship between another pro-inflammatory cytokine (TNF-α, tumor necrosis factor-alpha) and eGFR [19].

IL-6 is a glycoprotein consisting of 184 amino acids, which acts through the formation of a receptor complex on cell membranes. The receptor

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1st group (n = 154)</th>
<th>2nd group (n = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>(51.6 ± 13.9)</td>
<td>(57.9 ± 14.5) *</td>
</tr>
<tr>
<td>Gender, female — male</td>
<td>60.3% — 46.5%</td>
<td>39.7% — 53.5%</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>(133 ± 22)</td>
<td>(142 ± 24)*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>(86 ± 14)</td>
<td>(87 ± 15)</td>
</tr>
<tr>
<td>Heart rate, beat/min</td>
<td>(79 ± 14)</td>
<td>(80 ± 15)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>(28.3 ± 5.2)</td>
<td>(34.0 ± 3.6)</td>
</tr>
<tr>
<td>Interleukin-6, pg/ml</td>
<td>1.754 (0.494–3.226)</td>
<td>2.761 (1.400–6.495) *</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/l</td>
<td>69.3 (57.6–83.0)</td>
<td>115.9 (88.4–307.0) *</td>
</tr>
<tr>
<td>Serum Cystatin C, mg/l</td>
<td>0.980 (0.900–1.100)</td>
<td>1.815 (1.430–3.070) *</td>
</tr>
<tr>
<td>Estimated GFR, ml/min</td>
<td>77.6 (68.7–84.9)</td>
<td>59.9 (21.8–51.8) *</td>
</tr>
</tbody>
</table>

Notes. BP — blood pressure; GFR — glomerular filtration rate; n — the number of patients; * p < 0.05

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Interleukin-6, pg/ml</th>
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<tbody>
<tr>
<td></td>
<td>R = 0.119</td>
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<tr>
<td></td>
<td>R = 0.063</td>
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<tr>
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<td>R = 0.119</td>
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<td>R = 0.011</td>
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<td>R = 0.023</td>
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<td>R = 0.038</td>
</tr>
<tr>
<td></td>
<td>R = 0.144</td>
</tr>
</tbody>
</table>

Notes. IL — interleukin; GFR — glomerular filtration rate; R — correlation; P — reliability
complex consists of two parts: specific IL-6 receptor and non-specific CD130 transmembrane receptor [20, 21]. The latter is responsible for conducting the signal into the cell and consists of two identical subunits that form the bed for the IL-6 receptor. There are two types of specific IL-6 receptor: bound to the cell membrane (IL-6R) and soluble, freely circulating in the blood (IL-6Rs). Both receptors are activated when IL-6 is attached. It is believed that the sensitivity of these two types of receptors is quite high (0.5–2.0 nM) and almost the same [22, 23]. It was established that IL-6 increase is observed in obesity and type 2 diabetes mellitus [24]. Considering this, the proportion of patients that were overweight and had type 2 diabetes mellitus in our study was quite large. Previously, an IL-6 increase was shown to be associated with proliferation of vascular smooth muscle cells and increased production of platelet-derived growth factor [25, 26]. This probably explains the participation of pro-inflammatory cytokines in the occurrence of cardiovascular complications in CKD. We were not able to identify a correlation between IL-6 level and SBP (Table 3), although in the 2nd group, the values of the pro-inflammatory cytokine and SBP were significantly higher (Table 2). At the same time, there was a tendency for a close relationship between IL-6 level and DBP (Table 3).

The resulting interference between serum IL-6 and hemodynamic parameters is partly explained by the concept according to which IL-6 induces vasoconstriction and increases the activity of the sympathetic nervous system. In our previous work, we found that an increase in the concentration of proinflammatory cytokines (TNF-α) is associated with an increase in vascular stiffness [19]. Thus, we would like to note that the pathogenetic mechanisms of CKD progression involving proinflammatory cytokines, in particular IL-6, are extremely complex, diverse, and require further study.

Conclusions

1. In patients with CKD with an eGFR level below 60 ml/min, IL-6 increase is recorded.
2. In CKD, serum IL-6 level is closely related to the glomerular filtration rate and diastolic blood pressure.

Contribution of Authors
I. T. Murkamilov — writing of the text
Zh. A. Murkamilova, N. A. Redzhapova, Z. R. Rayimzhabanov — collection and processing of data
K. A. Aitbaev, V. V. Fomin, I. S. Sabirov, F. A. Yusupov — editing

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