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Serum Cystatin C and Neutrophil Gelatinase-Associated Lipocalin as Biomarkers of Glomerular and Tubular Kidney Damage in Patients with Chronic Glomerulonephritis and Saved Renal Function

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Abstract

Aim: to analyze the histological kidney features in patients with CGN and arterial hypertension (AH) and without it, to assess the relationship between serum CysC and NGAL and histological signs of glomerular and tubular kidney lesion in patients with CGN with saved renal function.
Methods: eighty one patients with CGN were enrolled in the study. Blood samples for biomarkers were collected. The diagnosis of "chronic glomerulonephritis" was defined by the clinical, laboratory data and renal biopsies. Patients were divided into 2 clinical groups: patients with AH and without AH. We used data of renal biopsies to analyze the signs of kidney glomerular apparatus and tubulo-interstitial tissue lesion in patients with CGN. Levels of serum CysC and NGAL were measured by ELISA kits.

Results: according to the results of renal microscopy 88% patients had mesangial proliferative glomerulonephritis, 7% – membranous nephropathy, 5% – membranous proliferative glomerulonephritis. Patients with CGN and AH have more severe histological glomerular and tubular lesion parameters than patients with CGN without AH. On the basis of rank correlation analysis we proved that serum cysC directly correlates with all indicators of glomerular apparatus kidney lesion in patients with CGN, strong direct relationship was found between the level of serum cysC and glomerulosclerosis ($r = 0.85$, $p < 0.05$). Level of serum NGAL directly correlates with indicators of tubulo-interstitial kidney tissue lesion in patients with CGN, strong direct relationship was found between the level of serum NGAL and interstitial fibrosis ($r = 0.65$, $p < 0.05$).
Conclusion: glomerulosclerosis has determined of the serum cysC level with the efficiency of 96.55%. Diagnostics of interstitial fibrosis by determining of the serum NGAL level is a highly sensitive and specific method, with the efficiency of 95.3%.

Keywords: cystatin C; NGAL; glomerulonephritis; pathology: diagnostics

Introduction

Chronic glomerulonephritis (CGN) is a serious problem in modern medicine due to the severity of the disease, worldwide prevalence, early morbidity and mortality of young patients. [2].

Renal biopsy remains one of the main diagnostic methods in modern nephrology. It allows to determine the nature of pathological changes, to predict the effectiveness of therapy, risk of adverse outcome and rate of renal function loss. One of the most important disadvantages of this study is invasiveness and well-defined indications to its conduction.

Today in practice, we use a variety of kidney damage markers, which reflect renal function and have predictive value. Among them, blood creatinine, glomerular filtration rate (GFR) and proteinuria. However, it is known that the level of creatinine is not able to adequately reflect slight changes in GFR and exposed external factors such as nutrition, body weight, gender, age and ethnic differences. Proteinuria is a marker with low specificity. The general lack of formulas for GFR calculating is their inaccuracy at normal or slightly reduced GFR values. That is why nowadays we have an increased interest in biological markers that allow estimating the activity and stage of renal process, assume the character of morphological kidney changes and monitor the effectiveness of treatment [1, 3, 6].

According to the literature, definition of cystatin C (CysC) and neutrophil gelatinase-associated lipocalin (NGAL) levels make it possible to determine the glomerular and tubular kidney lesions [3-6]. It gives grounds for using them as diagnostic indicators of glomerular apparatus and tubulo-interstitial tissue damage in patients with chronic glomerulonephritis [7]. The lack of reliable published data on this subject makes this assumption very promising.

Aim: to analyze the histological kidney features in patients with CGN and arterial hypertension (AH) and without it, to assess the relationship between serum CysC and NGAL and histological signs of glomerular and tubular kidney lesion in patients with CGN with saved renal function.

Design and Methods

Eighty one patients with CGN were enrolled in the study. The diagnosis of "chronic glomerulonephritis" was defined by the clinical, laboratory data and renal biopsies. Patients were divided into 2 clinical groups: patients with AH and without AH. The first group included 49 patients with CGN and AH, 34 (69%) men, 15 (31%) women, who had an average age 36.3±2.3 years, disease duration 87.1±9.8 months. Nephrotic syndrome with proteinuria (PU) above 3 g/l was observed in 8% of patients. Urinary syndrome with low PU and different severity of erythrocyturia was detected in 86% of patients, PU above 1 g/l – in 6%. The average daily rate of PU was 1.4±0.1 g/day. The second clinical group – 32 patients with CGN without AH, 20 (63%) men, 12 (37%) women, average age 38.7±7.5 years, disease duration 47.1±6.8 months. Urinary syndrome was manifested by low PU and different severity of erythrocyturia. In 66% of patients lab picture was defined as remission. Intermediate PU was 0.41±0.04 g/day. The control group consisted of 20 healthy persons, who were examined to clarify the standards of level markers. We have used data of renal biopsies to analyze the signs of kidney glomerular apparatus and tubulo-interstitial tissue lesion in patients with CGN.

To assess the glomerular apparatus degree destruction such parameters were defined as mesangial cellularity score, expansion of mesangial matrix (MM), presence of glomerulosclerosis, fibrocellular crescents, endocapillary hypercellularity, thickening of peripheral capillary loops and the glomerular basement membrane. Analysis of the tubulointerstitial tissue damage was performed on the following signs: dystrophic and necrotic changes in epithelial tubules, thickening of the tubular basement membrane, presence of cellular infiltration, interstitial fibrosis [2].

Serum concentration of both CysC and NGAL were measured by ELISA kits (BioVendor, Czech Republic).

We have evaluated the diagnostic accuracy of renal morphological changes on the level of serum biomarkers compared with biopsy by calculating operating performance tests, which include: diagnostic sensitivity, diagnostic specificity and diagnostic efficiency.

Statistics

All statistical analyses were performed in SPSS for Windows v. 7 17.0 (SPSS Inc., USA). The data was presented as mean and ±standard deviation, serum levels of markers were presented as median and 25%-75% confidence interval (CI). A calculated difference of $P<0.05$ was considered significant.

Results

The results of the main laboratory parameters of studied patients are presented in table 1.

Table 1 The basic laboratory parameters of patients with CGN (M±m)

The indicator	Patients with CGN and AH (n=49)	Patients with CGN without AH (n=32)	P-value
Hemoglobin, g/l	136.5±2,0	138.5±0.5	NS
Blood albumin g/l	42.8±0.7	44.1±0.2	NS
Blood cholesterol, mmol/l	5.6 ± 0.3	4.9±0.4	NS
Blood creatinine, mmol/l	101.9 ± 3.5	97.2 ± 2.2	NS
Blood urea, mmol/l	5.8 ± 0.2	5.9 ± 0.3	NS
GFR (Cockroft-Gault), ml/min/1,73/м^2	93.8 ± 2.8	94.5 ± 1.8	NS
GFR (CKD-EPI), ml/min/1,73/м^2	88.7 ± 2.2	91.3 ± 2.3	NS

Abbreviations: NS, not significantly; GFR, glomerular filtration rate.

According to the results of renal microscopy 88% patients had mesangial proliferative glomerulonephritis, 7% – membranous nephropathy, 5% – membranous proliferative glomerulonephritis. Histological picture of patients with CGN and AH was characterized by the presence of diffuse mesangial cellularity score (53%), diffuse expansion of MM (76%), presence of glomerulosclerosis (55%), fibrocellular crescents (22%), thickening of peripheral capillary loops (49%) and the glomerular basement membrane (94%), endocapillary hypercellularity (73%), dystrophic and necrotic changes in epithelial tubules of varying degree (98% and 31%), thickening of the tubular basement membrane (61%), presence of cellular infiltration (14%), interstitial fibrosis (100%). Microscopy data of patients with CGN without AH was differed from the previous group and had the features: 69% – focal mesangial cellularity score, 56% – focal expansion of MM, presence of glomerulosclerosis was confirmed only in 6% of biopsy samples, and interstitial fibrosis – in 13%, thickening of peripheral capillary loops – 28%, thickening of the glomerular basement membrane – 62%, endocapillary hypercellularity was determined in 19% of patients, dystrophic changes of epithelial tubules were defined in 31%,

necrotic – in 9%, thickening of the tubular basement membrane – in 28%, presence of cellular infiltration – in 9%, fibrocellular crescents were absent.
The analysis of morphological study by two groups showed the presence of significant differences, in patients with CGN and AH indicators of glomerular and tubular changes were more pronounced than in patients without AH.
We investigated the serum levels of markers in patients with CGN and control group. Results are presented in table 2.

The serum cysC level in patients with CGN was higher compared to the control group. Thus, in patients with AH cysC level was on 89% higher, in patients without AH – on 11% higher than in the control group. In patients with AH level of serum NGAL was in 2 times higher, in patients without AH – in 1.2 times higher than in the control group.

Table 2 Average serum levels of markers in patients with CGN and control group (Me: 25%-75%)

Biomarkers	Patients with CGN and AH (n=49)	Patients with CGN without AH (n=32)	Control group (n=20)
Serum cysC, ng/ml	2100.,0: 1403.7-2414.9	1163.6: 1033.1-1403,7	1092.3: 644.2-1187.7
Serum NGAL, ng/ml	6.2: 4.2-7.4	4,7: 3,8-7,4	2,8: 0.9-3.4

We determined serum cysC as a marker of glomerular kidney lesion, so a rank correlation analysis between cysC level and indicators of glomerular kidney damage in patients with CGN was conducted. Results are presented in table 3.

Table 3 Results of rank correlation analysis between serum cystatin C and indicators of glomerular kidney lesion in patients with CGN

The indicator of glomerular kidney lesion	Serum cysC	P-value
Expansion of mesangial matrix	r=+0.13	p>0.05
Thickening of peripheral capillary loops	r=+0.21	p<0.05
Thickening of the glomerular basement membrane	r=+0.30	p<0.05
Mesangial cellularity score	r=+0.32	p<0.05
Endocapillary hypercellularity	r=+0.37	p<0.05
Presence of fibrocellular crescents	r=+0.43	p<0.05
Glomerulosclerosis	r=+0.85	p<0.05

We found that level of serum cysC directly correlated with thickening of the glomerular basement membrane ($r = 0.30$, $p < 0.05$), peripheral capillary loops ($r = 0.21$, $p < 0.05$), mesangial cellularity score ($r = 0.32$, $p < 0.05$), presence of endocapillary hypercellularity ($r = 0.37$, $p < 0.05$) and fibrocellular crescents ($r = 0.43$, $p < 0.05$), glomerulosclerosis ($r = 0.85$, $p < 0.05$).

We evaluated diagnostic accuracy of glomerulosclerosis with serum cysC by calculating operating tests, such as diagnostic sensitivity, specificity and efficiency. Diagnostics of glomerulosclerosis was carried out by two methods in 81 patients with CGN: the first method – renal biopsy, which is considered the diagnostic standard, the second – the definition of serum cysC. Results are presented in table 4.

Table 4 Results of glomerulosclerosis diagnostics according to renal biopsy and serum cysC definition

The diagnostic method	Renal biopsy		
Serum cystatin C	Diagnostics	Glomerulosclerosis was present	Glomerulosclerosis was absent
	Glomerulosclerosis was present	27	0
	Glomerulosclerosis was absent	2	52

According to the table 4 we defined diagnostic sensitivity for glomerulosclerosis determining by the serum cysC, which was 93.1%, the diagnostic specificity – 100% diagnostic efficiency – 96.55%. Analysis of the operational characteristics of the glomerulosclerosis diagnostics by using serum cysC leads to the conclusion that this method is highly sensitive and specific, with the efficiency of 96.55%.

We suggested that serum NGAL is a marker of tubulo-interstitial kidney tissue lesion, so we conducted rank correlation analysis in patients with CGN. Results are presented in table 5.

We found that level of serum NGAL directly correlated with necrotic and dystrophic changes in epithelial tubules ($r = 0.27$, $r = 0.35$, $p < 0.05$), thickening of the tubular basement membrane ($r = 0.42$, $p < 0.05$) and interstitial fibrosis ($r = 0.65$, $p < 0.05$).

Table 5 Results of rank correlation analysis between serum NGAL and indicators of tubulo-interstitial kidney tissue lesion in patients with CGN

The indicator of tubulointerstitial kidney tissue lesion	Serum NGAL	P-value
Presence of cellular infiltration	r=+0.15	p>0.05
Necrotic changes in epithelial tubules	r=+0.27	p<0.05
Dystrophic changes in epithelial tubules	r=+0.35	p<0.05
Thickening of the tubular basement membrane	r=+0.42	p<0.05
Interstitial fibrosis	r=+0.65	p<0.05

We evaluated diagnostic accuracy of interstitial fibrosis with serum NGAL by calculating operating tests at the above technique. Results of the analysis are presented in table 6.

Table 6 Results of interstitial fibrosis diagnostics according to renal biopsy and serum NGAL definition

The diagnostic method	Renal biopsy		
Serum NGAL	Diagnosis	Interstitial fibrosis	
		presented	not determined
	Interstitial fibrosis was present	48	0
	Interstitial fibrosis was absent	5	28

According to the table 6 we defined diagnostic sensitivity for interstitial fibrosis determining by the serum NGAL, which was 90.6%, the diagnostic specificity – 100% diagnostic efficiency – 95.3%. Analysis of the operational characteristics of the interstitial fibrosis diagnostics by using serum NGAL leads to the conclusion that this method is highly sensitive and specific, with the efficiency of 95.3%.

Conclusions

1. Patients with CGN and AH have more severe histological glomerular and tubular lesion parameters than patients with CGN without AH.
2. On the basis of rank correlation analysis we proved that serum cysC directly correlates with all indicators of glomerular apparatus kidney lesion in

patients with CGN, strong direct relationship was found between the level of serum cysC and glomerulosclerosis.

3. The serum level of NGAL directly correlates with indicators of tubulo-interstitial kidney tissue lesion in patients with CGN, strong direct relationship was found between the level of serum NGAL and interstitial fibrosis ($r = 0.65$, $p < 0.05$).

4. Diagnosis of interstitial fibrosis might determine using serum NGAL level with the efficiency of 95.3%.

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