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Research Article

The Role of Chemokine Genes in the Formation of Terminal Stage of Chronic Renal Failure

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ABSTRACT

The data on the role of chemokine genes (+1931A/T CCL4, A/G CXCL11 (rs4512021), -403A/G CCL5, C/G CCL2 (rs2857657), -801G/A CXCL12) in the formation of terminal stage of chronic renal failure,in patients with chronic glomerulonephritis,is presented in the work. It was established, that the allele A CXCL11 (rs4512021) (OR = 1.65) was the marker for the development of terminal stage of chronic renal insufficiency, and the genotype GG CXCL11was a protective factor for the development of terminal stage of chronic renal failure (OR = 0.22).

Keywords: chronic glomerulonephritis, chemokine genes, genetic polymorphism, terminal stage of chronic renal failure.

INTRODUCTION

Chronic glomerulonephritis (CGN) is a multifactorial, genetically determined, immune-mediated renal glomeruli disorder, which is accompanied by the development of renal failure, hypertension, and can lead to death, caused by chronic renal failure (Buraczynska et al., 2010; Kamyshova et al., 2016).

At present, the involvement of genetic component in the formation, development and progression of renal pathologies, including chronic glomerulonephritis, is actively studied (Litovkina et al., 2014; Sorokina et al., 2016). The genes of renin-angiotensin-aldosterone system, the system of endothelial synthesis, as well as the genes of integral membrane proteins, tumor necrosis factors, interleukinsare mainly considered as candidate genesin nephrology (Buraczynska et al., 2010; Litovkina et al., 2014). According to the literature data (Anders et al., 2010), chemokineshave significant importance in the development of CGN, activation of which occurs after triggering the chain of immune-inflammatory reactions. Chemokines control the migration of different types of leukocytes, having receptors from the bloodstream to the tissues, foci of inflammation, autoimmune process, participate in activation and differentiation of leukocytes, angiogenesis, fibrogenesis. Ultimately, these processes can invariably cause a violation of the normal functioning of kidney tissue, its hardening (Litovkina et al., 2014). In this regard, chemokine genes can be considered as potential candidate genes of CGN (Chow et al., 2007). The analysis of literature data allows to note, that the implication of genetic factors of chemokines in the development of renal pathologies, including chronic glomerulonephritis, has not been sufficiently studied to date, and the results of available scientific studies are very few and fragmentary (Piotrowski et al., 2010; Bagci et al., 2015).

In accordance with the foregoing, the study of the role of polymorphic markers of chemokine genes ((+ 1931A/T *CCL4*, A/G *CXCL11* (rs4512021), -403A/G *CCL5*, C/G *CCL2*

(rs2857657), -801G/A *CXCL12*)) in the formation of terminal stage of chronic renal failure, in patients with chronic glomerulonephritis, was carried out in this work.

MATERIALS AND METHODS

The studied sample included 700 people: 238 patients with chronic glomerulonephritis and 462 persons were in the control group. Formation of samples of patient and control group was carried out using a continuous method, in the period from 2009 to 2011. The samplesincluded persons of Russian nationality, who were natives of the Central Chernozem Region of the Russian Federation, and were not related to each other. The investigated group of patients with CGN and the control group are comparable by gender, age characteristics, place of birth and nationality.

Patients were included in the appropriate group only after making the diagnosis, confirmed with the help of clinical and laboratory-instrumental methods of examination. Clinical and laboratory examination of patients was carried out on the basis of Nephrology Department of the Belgorod Regional Clinical Hospital of St. Joasaph. All patients signed an informed consent for inclusion in the research and the use of obtained data. Ethical principles of the World Medical Association Declaration of Helsinki were adhered in the course of work with the surveyed persons. The control group included individuals without kidney disease and hypertension.

Persons with hypertension and with a history of diabetes (or identified during the examination) were excluded from the group of patients.

DNA samples were the material of the study. They were recovered from the whole venous blood, taken from the median cubital vein of proband, using the phenol-chloroform extraction method (Miller et al., 1988). Recovered DNA was used for performing the polymerase chain reaction (PCR) of DNA synthesis, using standard oligonucleotide primers and probes. Subsequent analysis of polymorphisms was carried out by the method of Taq-Man probes detection, with the help of real-time PCR.

The analysis of association of alleles and genotypes of the studied DNA markers, with the formation of terminal stage of chronic renal failure, was carried out using conjugation tables 2x2, with the calculation of the criterionχ^2, with the Yates correction for continuity and odds ratio (OR), with 95% confidence intervals (CI). In the process of performing of multiple comparisons, with the aim to minimize errors of the first kind,connected with obtaining of false positive results, the Bonferronicorrection(p_{cor}) was used. Statistical calculations were carried out using the program "STATISTICA 8.0".

RESULTS AND DISCUSSION

All patients with CGN and individuals in the control group were typed with five molecular-genetic markers of chemokines: regulator of activity of T-cells normal expression (-403G/A *CCL5*), growth-promoting factor of β-cells precursors (-801G/A *CXCL12*), monocyte chemoattractant protein 1 (C/G *CCL2*, rs2857657), interferon inducible α chemoattractantofT-cells (A/G *CXCL11*, rs4512021), macrophage inflammatory protein 1β (+ 1931A/T *CCL4*).

The main characteristics of the studied group of patients with chronic glomerulonephritis and control group are presented in Table 1. The control group is fully comparable with the sample of patients with CGN, according to these characteristics ($p > 0.05$).

In the process of comparative analysis of the frequencies of alleles and genotypes of the investigated loci, no statistically significant differences were found between CGN patients and the control group ($p > 0.05$).

Further, we carried out a comparative analysis of the frequency distribution of alleles and genotypes of chemokine genes among patients, who reached the terminal stage of chronic renal failure (n = 71), and in the control group. The results of this study are presented in Table 2. It was found, that among patients with terminal stage of chronic renal failure, the concentration of genotype GG *CXCL11* (rs 4512021) is 5.08%, that is almost 4 times less, than the corresponding value in the control group

(19.69%, OR = 0.22, 95% CI 0.14-0.75, χ^2 = 6.57, p = 0.01,p_{cor}= 0.03).

Similar data were obtained on the alleles frequencies of the locus A/G *CXCL11*. Among patients with terminal stage of chronic renal failure, the frequency of the allele G *CXCL11*was 32.20%, whereas in the control group the prevalence of this marker was 43.96% (OR = 0.61, 95CI% 1.08-2.54, χ^2 = 5.42, p = 0.02). Revealed relationship between these genetic variants,with the formation of terminal stage of chronic renal failure in CGN patients, can be explained from the standpoint of their biomedical effects. It is known, that certain allelic variants of genes (usually "mutant" alleles) cause the increased production of corresponding cytokines (Sorokina et al., 2016), which will be clinically manifested by their more pronounced biomedical effects. According to the literature data, individuals with 4 and 5 stages of chronic kidney disease (CKD) have higher concentration of CXCL11 in blood, than healthy individuals and patients with 1-3 stages of CKD (Lebherz-Eichinger et al., 2014). Interferon inducible α-chemoattractant of T-cells is the most important chemotactic factor for T-lymphocytes,itactivates Th1CD4 T-cells, NK cells, monocytes in the focus of inflammation (Teramoto et al., 2008). This can play an important role in maintaining of chronic inflammatory process in the kidney, causing a decline in its functioning, and also it can lead to the end-stage of chronic renal failure.

CONCLUSION

As a result of the study, it was established, that the allele A of interferon inducible α-chemoattractant of T-cells (rs4512021) (OR = 1.65) could be considered as a marker for the development of terminal stage of chronic renal failure in patients with chronic glomerulonephritis, and the genotype GG *CXCL11*was a protective factor of development of terminal stage of chronic renal failure (OR = 0.22).

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Tables

Table 1.Characteristics of the subjects from the case and control groups.

Characteristics	Cases	Controls
Total	238	462
Males	53.4%	53.7%
Females	46.6%	46.4%
Age, yrs	39.58 ±14.58	42.12 ± 5.19
Weight, kg	63.4 ± 2.1	66.4 ± 2.0
Height, cm	165.4 ± 3.4	169.5 ± 1.9

Table 2. Distribution of genotypes of polymorphic loci of chemokines among patients with chronic glomerulonephritis with terminal stage of chronic renal failure and in the control group

Polymorphism		Control group (n=462)		CGN patients with terminal stage of chronic renal failure (n=71)		OR (95%CI)	χ2 (p)
Locus	Genotype	n	%	n	%		
+1931A/T*CCL4*	+1931AA	31	6,87	4	5,88	0,85 (0,25-1,03)	χ^2=0,02; p=0,96
	+1931AT	184	40,80	37	54,41	1,73 (1,01-2,98)	χ^2=3,94; p=0,05
	+1931TT	236	52,53	27	39,71	1,65 (0,08-2,54)	χ^2=3,27; p=0,07
A/G *CXCL11* (rs 4512021)	AA	142	31,76	24	40,68	1,52 (0,84-2,74)	χ^2=1,79; p=0,18
	AG	217	48,55	32	54,24	1,25(0,70-2,24)	χ^2=0,47; p=0,49
	GG	88	19,69	3	5,08	0,22 (0,14-0,75)	χ^2=6,57 p=0,01
-403A/G *CCL5*	-403GG	286	67,29	26	58,33	0,69 (0,38-1,23)	χ^2=1,50; p=0,22
	-403GA	126	29,65	35	40,00	1,58 (0,87-2,55)	χ^2=2,17; p=0,14
	-403AA	13	3,05	24	1,67	0,56 (0,02-4,24)	χ^2=0,01; p=0,89
C/G *CCL2* (rs2857657)	CC	313	68,34	44	64,71	0,86 (0,49-1,55)	χ^2=0,14; p=0,71
	GC	133	29,04	23	33,82	1,12 (0,61-2,03)	χ^2=0,06 p=0,79
	GG	12	2,62	1	1,47	1,31 (0,19-6,57)	χ^2=0,01; p=1,00
-801G/A *CXCL12*	- 801GG	320	71,11	47	68,12	0,85 (0,48-1,50)	χ^2=0,21; p=0,64
	- 801GA	120	26,67	20	28,99	1,25 (0,70-2,20)	χ^2=0,44; p=0,50
	- 801AA	10	2,22	2	2,89	0,56 (0,02-4,20)	χ^2=0,02; p=0,88

Notes: n, sample size; CGN, chronic glomerulonephritis; OR, odds ratio; 95%CI, 95% confidence interval; P values were calculated using the χ^2 test.