ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Chemokines Genetic Variants are Associated with Parameters of Humoral Immunity of Patients with Chronic Glomerulonephritis.

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ABSTRACT

The authors researched associations of chemokines genes polymorphisms (+1931A/T *MIP1β*, A/G *I-TAC* (rs4512021), -403A/G *RANTES* , C/G *MCP1*(rs2857657), -801G/A *SDF1*) with parameters of humoral immunity of 238 patients with chronic glomerulonephritis and 462 individuals of a control group. It has been revealed that the marker of increased level of IgG under chronic glomerulonephritis is +1931TT *MIP1β*, and high concentrations of IgA during aggravation period are associated with - 801AA, - 801GA *SDF1*.

Keywords: chronic glomerulonephritis, humoral immunity, immunoglobulins, chronic renal failure, genetic polymorphism, chemokines.

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ISSN: 0975-8585

INTRODUCTION

Among parenchymatous kidney diseases chronic glomerulonephritis lies in a principal place. Among nephrological diseases chronic glomerulonephritis (CGN) takes more than 35% [1, 2]. It is evident that this is the most often-met type of pathologic process in kidneys which is one of the most widespread causes of chronic renal failure (which requires haemodialysis and kidney transplantation for curing) [3]. Prevalence rate and chronic disease end-stages incidence rate increase consistently in different regions of the world [4, 5].

According to experimental and clinical data, an important role in CGN progress is played by chemokines [6]. Clinical genetic works dedicated to molecular genetic aspects of CGN are rare in Russia and touch mainly the spectrum of vasoactive hormones genes, tumour necrosis factors, integral membrane proteins and some interleukins [7, 8]. Researches of the role of chemokines genes polymorphous markers regarding CGN have not been carried out yet. Whereas chemokines, being chemoattractants, play an important role in the progress of inflammatory reactions in an organism and also immuno-inflammatory processes under chronic glomerulonephritis [9].

Due to this, lately researchers take an increasing interest in chemokines genes polymorphous markers under kidney diseases, classifying them as possible glomerulopathies risk genetic factors. In pursuance of the foregoing, in this work we have carried out the investigation of chemokines genetic polymorphisms (+1931A/T *MIP1β*, A/G *I-TAC* (rs4512021), -403A/G *RANTES* , C/G *MCP1*(rs2857657), -801G/A *SDF1*) associations with parameters of humoral immunity of patients with chronic glomerulonephritis.

MATERIALS AND METHODS

We carried out the analysis of chemokines genes polymorphisms of 700 persons: 238 patients with chronic glomerulonephritis (average age 39.58±14.58 years old, varied from 15 to 76 years old) and 462 individuals of a control group (42.20±6.28 years old, varied from 18 to 79 years old, $p>0.05$). The sample of patients and the control group included individuals of Russian ethnicity, natives of Central Black Earth region of Russia, being not related to each other. Patients were included into the patients group only after diagnosing of the disease and confirming the diagnosis with the help of clinical and laboratory instrumental methods of examination. Clinical laboratory examination of patients was carried out housed by nephrology department of Belgorod region clinical hospital.

During the period of blood sampling (questioning), when the patients were passing through hospital treatment for the term from two to four weeks, level of immunoglobulins IgA, IgM, IgG was estimated via enzyme-linked immunoassay (ELISA), using blood serum samples and standard sets according to a manufacturer's manual.

General clinical examination of patients included careful study of medical history, physical examination, complete blood count, common urine analysis with microscopic evaluation of urinary sediment, estimation of daily proteinuria, research of biochemical parameters of blood (total protein, albumin, protein fractions creatinine, uric acid, urea, cholesterol).

Exclusionary criteria for the group of patients with CGN were diabetes mellitus (in anamnesis or found as a result of examination), hypertension.

As the research material we used venous blood – 8-9 ml from a proband's median cubital vein. Extraction of genomic DNA from peripheral blood was performed with the help of standard methods [10].

Analysis of all loci was carried out with the help of the method of DNA synthesis polymerase chain reaction, using standard oligonucleotide primers and probes [11, 12, 13].

Genotyping of DNA markers (+1931A/T *MIP1β*, A/G *I-TAC* (rs4512021), -403A/G *RANTES* , C/G *MCP1*(rs2857657), -801G/A *SDF1*) was carried out via analysis of alleles discrimination with the help of the method of Tag Man probes (Tag Man). Associations of alleles and genotypes of the studied DNA-markers with arterial hypertension of the patients with chronic glomerulonephritis were estimated with the help of analysis of cross tables 2x2 calculating the criterion $\chi 2$ with Yates correction for continuity and odds ratio (OR) with

95% confidence intervals (CI). For studying of parameters of glomerular filtration rate and creatinine level we used median (Me) and interquartile range (Q25-Q75), and for comparative analysis we used Mann-Whitney test. ("STATISTICA 6.0").

RESULTS

We examined 238 patients with chronic glomerulonephritis and 462 individuals of a control group. Main characteristics of the examined patients with chronic glomerulonephritis and the control group are presented in table 1. It should be noted that the group of CGN patients had much higher levels of IgA and IgG than the control group ($p<0.001$).

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

Characteristics	Cases	Controls
Total	238	304
Males	127 (53.4%)*	125 (51.86%)
Females	111 (46.6%)*	116 (46.64%)
Age, yrs	39.58 ± 14.58*	42.20 ± 6.28
Weight, kg	63.4 ± 2.1*	67.4 ± 1.7
Height, cm	165.4 ± 3.4*	168.6 ± 2.7
SBP, mm Hg	148.4 ± 26.5**	128.1 ± 4.4
DBP, mm Hg	92.7 ± 14.0**	82.2 ± 2.0
Creatinine, µmol/L	337.2 ± 44.1**	130.4 ± 7.8
GFR, ml/min	28,2±1,8	81,6±3,4
IgA, g/l	3,75±0,14**	2,98±0,15
IgM, g/l	2,95±0,13	2,80±0,20
IgG, g/l	18,85±0,56**	12,42±0,73

Note: *p>0.05; **p<0.001.

In the course of analysis of immunoglobulins concentration of the CGN patients, depending on presence and intensity of chronic renal failure (CRF), it was found that, firstly, CGN patients without CRF differ from the control group only in a high level of IgG (20.28±0.85g/l against 12.42 ±0.74g/l in the control group, $p< 0.001$). Secondly, CGN patients with CRF differ from the control group considerably in the level of analyzed immunoglobulins: concentration of IgA (3.96±0.18 g\l) and IgG (16.94 ±0.69g/l) is much higher, and content of IgM (2.43 ±0.11g/l) is lower than in the control group.

We found connections of genetic polymorphisms +1931 A/T *MIP1β* and -801G/A *SDF1* with the immunoglobulins level of the CGN patients. Individuals with the genotype +1931 TT *MIP1β* had the G immunoglobulin concentration which was equal to 22.02±2.02 g/l and was credibly higher than of patients with genotypes +1931 AA and +1931 AT *MIP1β* (18.53±0.59 g/l, p=0.05).

We revealed differences in the nature of associations of chemokines molecular genetic markers with immunoglobulin concentration in case of patients with different degrees of the process activity (disease-free survival and aggravation) and in case of patients depending on presence of chronic renal failure (CRF). Patients with chronic glomerulonephritis aggravation have a high level of IgA (4.99±0.67 g/l) if they have genotypes – 801AA and - 801GA *SDF1* , as compared to patients with a genotype - 801 GG *SDF1* (2.95±0.27 g/l, p=0.01). We revealed connections of genetic polymorphism +1931A/T *MIP1β* with concentration of IgG in the group of patients without CRF: individuals with a genotype +1931TT *MIP1β* had G immunoglobulin concentration equaled to 25.58±1.62 g/l and it was 1.3 times higher than of the patients with genetic markers +1931AA and +1931AT *MIP1β* (19.86±0.89 g/l, p=0.02).

SPECULATION

We found connection of the genotype +1931 TT *MIP1β* with a high level of Ig G of the CGN patients. Macrophage inflammatory protein (*MIP 1β),* along with chemoattractant properties, induces adherence of human's circulating lymphocytes to endothelium [14] and due to this play an important role in the inflammatory cytokines cascade progress in an organism.

At the same time, it has been found that the polymorphous genetic marker -801 G/A *SDF1* is associated with humoral immunity peculiarities of the CGN patients. Patients with genotypes -801 AA and -801 GA *SDF1* have a high level of immunoglobulin A in the aggravation period.

SDF1, being a pre-B-cells growth promotant, conditions intensification of homing of some types of stem cells into affected organs (including kidneys), proliferation stimulation, intensification of adhesion and motility of cells in a pathologic focus [15], and that plays an important role in the progress of immuno-inflammatory reactions in glomerular apparatus of kidneys.

CONCLUSION

Thus, the work's results allow us to conclude that the concentrations of A and G immunoglobulins of the patients are 1.3 – 1.5 times higher than the control group's parameters ($p<0.001$) and are interconnected with the chronic renal failure progress. The marker of an increased level of IgG under chronic glomerulonephritis is +1931TT *MIP1β*, and high concentrations of IgA in the aggravation period are associated with - 801AA, - 801GA *SDF1*.

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