GENETICS

Plasminogen-1 Activator Inhibitor Gene 4G/5G Promotor Polymorphism and Blood Level of Its Protein Product in Hemorrhagic Fever with Renal Syndrome

A. A. Baygildina, D. V. Islamgulov*, and I. R. Minniakhmetov*

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The dynamics of plasma level of plasminogen-1 activator inhibitor (PAI-1) and frequency distribution of PAI-1 gene polymorphic locus 4G/5G genotypes and alleles were studied in male and female patients of different age with hemorrhagic fever complicated by renal syndrome of different severity. A significant elevation of PAI-1 level was recorded during fever in adult patients of mature age groups I and II with medium severe and severe uncomplicated disease, the elevation being followed by a significant reduction, except the oliguric and polyuric periods of medium severe form. In complicated disease, the concentration of PAI-1 was low during fever in patients of mature age group I, after which it increased significantly until the period of diuresis recovery; in patients of mature age group II it remained low, except the polyuria period. No appreciable age- or gender-related differences in frequency distributions of PAI-1 gene polymorphic locus 4G/5G genotypes and alleles in patients with disease of different severity were found; no differences from the control group by these parameters were revealed. The dynamics of PAI-1 plasma level in different forms of hemorrhagic fever with renal syndrome were not genetically determined and represented an adequate metabolic response to endotheliotropic virus.

Key Words: *plasminogen-1 activator inhibitor (PAI-1); PAI-1 gene 4G/5G polymorphism; hemorrhagic fever with renal syndrome*

Plasminogen-1 activator inhibitor (PAI-1) belongs to the serine proteinase superfamily and is the main antagonist of plasminogen tissue activator and urokinase stimulating plasminogen and thus promoting fibrinolysis. The synthesis of PAI-1 is stimulated by endotoxin, proinflammatory cytokines (IL-1β and TNF-α), and thrombin [8] and increases significantly in sepsis and extensive thrombosis [12]. The PAI-1 gene is located in the long arm of chromosome 7 (7q22) [10]. The main insertion/deletion polymorphic locus of PAI-1 gene 4G/5G is located in the promotor region (in position -675 b. p. from the start codone) [6]. Guanine insertion (5G allele) leads to the formation of an extra transcription repressor binding site, which results in reduced total activity of PAI-1 in the blood [5] and hence, hetero- and homozygotic carriers of 4G allele have higher plasma levels of the inhibitor than carriers of 5G allele, which is fraught with a high risk

Department of Biological Chemistry and Organic Biochemistry, Bashkir State Medical University, the Russian Ministry of Health, Ufa; *Laboratory of Human Molecular Genetics, Institute of Biochemistry and Genetics, Ufa Research Center, the Russian Academy of Sciences, Russia. ***Address for correspondence:*** baigildinaasia@mail.ru. A. A. Baygildina

of thrombus formation [9]. Expression of PAI-1 is found mainly in endotheliocytes [14] and also in hepatocytes, monocytes, macrophages, adipocytes, *etc.* [13]. The endothelium is not only the main producer of this substance, but also offers its surface for many hemostasiological reactions. Due to its unique location between the blood and tissue, the endothelium is the first that is involved in the pathogenesis of many diseases, including hemorrhagic fever with renal syndrome (HFRS), because its agent (hantavirus) is highly tropic to vascular endothelium. The disease is characterized by fever, total intoxication, peculiar renal involvement, development of the thrombohemorrhagic syndrome up to development of the DIC syndrome. In Russia, HFRS is the leading zoonotic infection which ranks the first among the natural focal infections [3]. Associations of PAI-1 4G/5G gene polymorphism with a high risk of thrombosis in diseases linked with endotheliopathy development have been described [7,11], but the effect of genetic factors on the development of endothelial abnormalities in infectious diseases has never been analyzed.

We evaluated associations of PAI-1 gene polymorphic locus 4G/5G with the levels of secretion of the respective protein product in various periods of HFRS of different severity in patients of different age and genders.

MATERIALS AND METHODS

The study was carried out in 348 patients with HFRS serologically confirmed by indirect fluorescent antibody method (284 men and 64 women aged 22-60; mean age 39.1±3.6 years), treated at Clinical Hospital for Infectious Diseases No. 4, Ufa, and at Hemoperfusion Department of G. G. Kuvatov Republican Clinical Hospital in 2003-2009. Comparative analysis of frequency distribution of PAI-1 gene polymorphic locus 4G/5G genotypes and alleles was carried out in 286 patients (229 men, 57 women); plasma levels of PAI-1 were measured in 62 patients (55 men and 7 women). No patients with a history of essential hypertension, cardiovascular diseases, diabetes mellitus, malignant tumors, or hepatorenal diseases participated in the study. The severity of HFRS was evaluated using B. Z. Sirotin's classification [4]. Medium severe form was diagnosed in 201 patients (53.9%), severe without complications in 110 (29.5%), severe with complications in 62 (16.6%) patients. The patients were distributed into age groups in accordance with the Age Periods approved by the International Workshop on Age Periods (Moscow, 1965). A total of 165 patients were referred to mature age period I (22-35 years), 183 patients to mature age period II (36-60 years for men and 36-55 years for women). Control group consisted of 105 healthy volunteers of similar genders and age. Plasma concentrations of PAI-1 antigen were measured by enzyme immunoassay with Technoclone kits. Light absorption was recorded using Bench mark microplate reader (Bio-Rad). The DNA was isolated from the peripheral blood by phenol-chloroform extraction. The PAI-1 gene 4G/5G polymorphic variant was analyzed by PCR synthesis of DNA with subsequent analysis of restriction fragment lengths by enzymatic hydrolysis using BseLI restriction endonucleases (Fermentas) [7]. Amplification results were evaluated by electrophoresis in 2% agarose gel with subsequent staining by ethidium bromide and visualization in transmitting ultraviolet light.

The results of measurements of PAI-1 antigen were processed using Statistica 7.0 and SPSS 13 software. The median, interquartile interval (procentiles 25 and 75), maximum and minimum values were evaluated for PAI-1. The significance of differences between the means for groups was evaluated by Mann–Whitney *U* test with Bonferroni correction. The frequencies of genotypes and alleles were compared using Pierson χ^2 test. The critical level of zero statistical hypothesis p significance was assumed at 0.05.

RESULTS

Plasma levels of PAI-1 antigen in HFRS patients depended on the disease period and severity and patient's age. Changes in the antigen concentrations in medium severe and severe uncomplicated forms of patients of mature age periods I and II had a similar wave-like dynamics with peaks during fever and minimum during diuresis recovery. More significant differences were characteristic of the medium severe form in both age groups (Figs. 1, 2).

In both forms of the disease, statistically high plasma levels of the antigen during fever (297.1 [240.4; 552.5] and 296.7 [240.4; 333.8] ng/ml, respectively, *vs.* 204.2 [188.8; 263.3] ng/ml in the control) reduced by the oliguria period (174.4 [136.7; 205.5] ng/ml, $p>0.05$, and 154.7 [125.1; 173.6] ng/ml, $p<0.05$). This was followed by a repeated elevation during polyuria (to 237.8 [200.0; 308.6] ng/ml, $p>0.05$, and 153.6 [120.5; 211.3] ng/ml, $p<0.05$) and by another statistically significant decrease during diuresis recovery (154.7 [125.1; 173.6] and 147.8 [96.5; 195.3] ng/ml, respectively). The time course of blood concentrations of PAI-1 in the two age groups differed in case of complications (DIC syndrome, infectious toxic shock, acute respiratory failure, acute erosive gastritis, *etc.*). In the younger patients (mature age I), a significantly low level of the inhibitor during fever (75.5 [63.8; 78.8] ng/ml, $p<0.05$) was followed by its elevation till the period of diuresis recovery (273.8

[216.8; 557.5], $p<0.05$). In the older patients (mature age I) the concentration of the antigen remained low throughout the disease, except the oligo-anuria period.

Presumably, the first generation cytokines IL-1β and TNF-α stimulated the production of PAI-1 during fever and subsequent inhibition till diuresis recovery (high production was more pronounced in severe uncomplicated form). The dynamics of blood concentrations of these cytokines in various forms of HFRS was similar to that of PAI-1 [1]. The low expression of the inhibitor gene provided the maintenance of normal blood rheology throughout the disease. Different dynamics of antigen concentration in younger patients (mature age I) with severe complicated HFRS could not be explained solely within the framework of the cytokine stimulation concept, because the development of the DIC syndrome, infectious toxic shock, and other complications and an extensive involvement of the endothelium [2] with the development of endotheliopathy could be paralleled by involvement in the pathogenesis of other factors regulating the fibrinolysis activity, aimed, for example, at stimulation of the clot lysis because of high thrombogenic activity of vascular walls because of exposure of the subendothelial layer. Under these conditions the mechanisms of compensatory stimulation of PAI-1 gene expression seemed to be triggered as early as during fever (despite the intense cytokine stimulation), as a result of which plasma levels of its protein product increased. In older patients (mature age II) this compensatory mechanism was presumably not triggered because of certain age-associated structural and functional changes in the inner lining of the vessels, responsible for its incompetence as a producer of fibrinolysis factors.

Since genetic variability of genes encoding the main components of fibrinolysis system is essential for the course and outcome of infectious diseases [9], including HFRS, we analyzed association of PAI-1 gene insertion/deletion 4G/5G polymorphism with the disease severity and development of complications in HFRS patients of different age and gender. The genotype frequency distribution in all the studied samples corresponded to the Hardy–Weinberg distribution. Comparative analysis of the gene's polymorphic locus genotype and allele frequency distribution showed no appreciable differences between the groups of patients with different severity of HFRS and in comparison with the control group (Table 1).

The frequency distribution of 4G/4G genotype was similar in the control and in patients with medium severe, severe uncomplicated, and severe complicated HFRS (32.5, 31.8, 27.6, and 24.4%, respectively). The heterozygotic 4G/5G genotype was somewhat more incident in patients with the severe complicated form (66.7%) than in medium severe (60.9%), severe uncomplicated HFRS (54.6%), and in the control group (48.75%). The frequency of homozygotic 5G/5G genotype was lower (8.9%) in patients with the complicated form than in medium severe (11.49%) and severe uncomplicated form (13.64%), *vs.* 18.75% in donors.

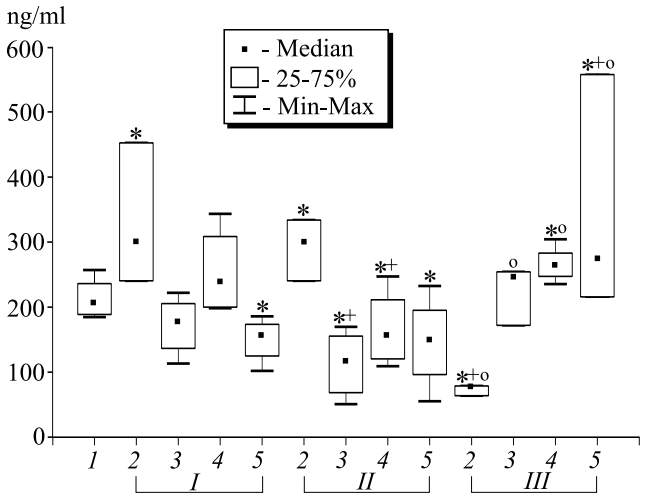

Fig. 1. Plasma concentration of PAI-1 antigen in HFRS patients of mature age I with disease of different severity receiving basic drug therapy. Here and in Fig. 2: *1*) control; *2*) fever; *3*) oliguria; *4*) polyuria; *5*) diuresis recovery. *I*) medium severe form; *II*) severe uncomplicated; *III*) severe complicated. p=0.05 *vs.* *control, +medium severe form, °severe uncomplicated form.

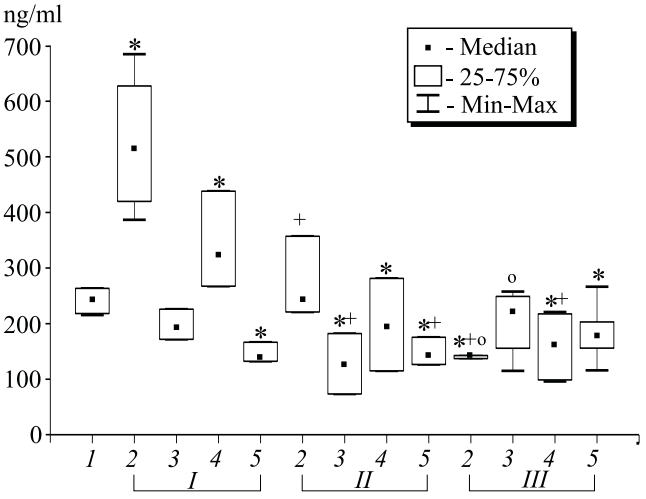

Fig. 2. Plasma concentrations of PAI-1 antigen in HFRS patients of mature age II with disease of different severity, receiving basic therapy.

Comparative analysis of frequency distribution of 4G/5G polymorphic locus genotypes and alleles showed no appreciable differences between the patients of different age groups (Table 2) and genders (Table 3).

The frequency distribution of 4G/4G, 4G/5G, and 5G/5G genotype was similar in HFRS patients of different age groups (29.1, 57.2, 13.6% and 30.8, 56.7, and 12.5%, respectively).

TABLE 1. Frequency Distribution of PAI-1 Gene 4G/5G Polymorphic Locus Genotypes and Alleles in HFRS Patients with Disease of Different Severity

Disease form		Genotypes			Alleles	
		4G/4G	4G/5G	5G/5G	4G	5G
Control	*n*	26	39	15	91	69
	pi±*Sp*	32.50±5.24	48.75±5.59	18.75±4.36	56.88±3.92	43.13±3.92
Medium severe	*n*	49	84	21	182	126
	pi±*Sp*	31.82±3.80	54.55±4.01	13.64±2.77	59.09±2.80	40.91±2.80
Severe uncomplicated	*n*	24	53	10	101	73
	pi±*Sp*	27.59±4.80	60.92±5.23	11.49±3.42	58.05±3.74	41.95±3.74
Severe complicated	*n*	11	30	4	52	38
	pi±*Sp*	24.44±6.41	66.67±7.03	8.89±4.24	57.78±5.21	42.22±5.21

Note. Here and in Tables 2, 3: *n*: absolute number of genotypes (alleles); *pi*: frequency; *Sp*: error in *pi*.

TABLE 2. Frequency Distribution of PAI-1 Gene 4G/5G Polymorphic Locus Genotypes and Alleles in HFRS Patients of Different Age

Mature age periods		Genotypes			Alleles	
		4G/4G	4G/5G	5G/5G	4G	5G
Period I	*n*	41	72	24	135	99
	pi±*Sp*	29.09±4.30	57.27±4.72	13.64±3.27	57.73±3.33	42.27±3.33
Period II	*n*	46	78	25	200	138
	pi±*Sp*	30.83±4.22	56.67±4.52	12.50±3.02	59.17±3.17	40.83±3.17

TABLE 3. Frequency Distribution of PAI-1 Gene 4G/5G Polymorphic Locus Genotypes and Alleles in HFRS Patients of Different Genders

Gender		Genotypes			Alleles	
		4G/4G	4G/5G	5G/5G	4G	5G
Females	*n*	17	25	15	38	32
	pi±*Sp*	25.0±8.2	57.14±9.35	17.86±7.24	53.53±6.66	46.43±6.66
Males	*n*	71	124	34	297	205
	pi±*Sp*	30.69±3.25	56.93±3.48	12.38±2.32	59.16±2.45	40.84±2.45

The homozygotic 4G/4G genotype was somewhat more incident in male patients with HFRS (30.7%) than in females (25%), while the homozygotic 5G/5G genotype predominated in women (17.9 and 12.4%, respectively). These differences in the distribution of homozygotic genotypes were statistically negligible and could be due to the little number of female patients. The frequency distribution of heterozygotic 4G/5G genotype in male and female patients with HFRS was comparable (57.1 and 56.9%, respectively).

The detected differences in frequency distribution of genotypes and alleles are statistically negligible. This indicated that the 4G/5G insertion/deletion polymorphism in PAI-1 gene was not associated with

HFRS severity and complications or with patients' age and gender. Hence, differences in the plasma levels of the inhibitor over the course of the disease in patients of different age could be regarded as quite an adequate metabolic response to the primary challenge with the endotheliotropic virus, HFRS agent.

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