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THE ROLE OF VITAMIN D METABOLISM DISORDERS IN THE PATHOGENESIS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME

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The aim of this work was to study the levels of 25 (OH) vitamin D (calcidiol - CD) and 1.25 $(OH)_2$ vitamin D (calcitriol - CT) in the blood of patients with hemorrhagic fever with renal syndrome (HFRS) depending on the severity, the period of the disease, and elucidation of the role of vitamin D metabolism disorders in the pathogenesis of HFRS.

Patients and methods. 114 patients with HFRS, aged 18–55 years (average age 37.4 ± 2.6 years) were under the supervision. Among them 53 patients were with moderately severe form, 61 - with severe one. The determination of serum calcidiol and calcitriol levels was carried out by high performance liquid chromatography. Determination of the level of cytokines (INF-γ, TNF-α, IL-2) was carried out by the enzyme immunoassay using reagents of Vector-Best CJSC (Novosibirsk).

Results. The lowest concentration of calcitriol and calcidiol in the blood serum is observed in the oliguric phase with moderately severe and severe forms

of HFRS. The more severe the disease, the more evident the deficiency of vitamin D active metabolites is. The study of cytokines in these patients revealed a sharp increase in TNF-α, a monotonously low level of IFN -γ, and a decrease in IL-2 in the febrile and oliguric phases. A reliable direct correlation between calcitriol and IL-2, as well as between calcitriol and INF-γ, and a significant inverse correlation between the concentration of calcitriol and the level of TNF-α were revealed.

Conclusions. Deficiency of vitamin D active metabolites may be one of the mechanisms contributing to the appearance of hypocalcemia affected hypocalceuria in the midst of HFRS. The presence of correlations of calcitriol with IL-2, INF-γ, and TNF-α indicates a possible role of deficiency of the vitamin D active metabolite in the development of immunological disorders in HFRS.

Key words: *hemorrhagic fever with renal syndrome, calcidiol, calcitriol, cytokines.*

Hemorrhagic fever with renal syndrome (HFRS) is the leading natural focal infection in the Russian Federation and ranks first in the regional pathology of the Republic of Bashkortostan (RB). Prevalence, high rates of morbidity accompanied by a long period of temporary disability, lack of effective and affordable specific means of treatment and prophylaxis determine the high social and medical significance of HFRS in the Republic of Bashkortostan. A significant place among the metabolic disorders that occur during HFRS is occupied by imbalances in vitamins, macro- and microelements [1-3]. According to the scientific literature, HFRS significantly affects the level of calcium in the blood. However, hypocalcemia during the oliguric phase of HFRS cannot be explained by increased excretion of calcium in the urine, since hypocalceuria is also observed during this phase. [4]. Studies of vitamin D levels as one of the regulators of phosphorus - calcium metabolism in HFRS have not been previously conducted. At the same time, it is the kidneys where vitamin D

active metabolite is formed - calcitriol, which not only regulates the level of calcium in the blood, but also has an immunotropic effect, while one of the main links of the pathogenesis is a violation in the immunological status in HFRS.

The aim of this work was to study the levels of 25 (OH) vitamin D (calcidiol - CD) and 1.25 $(OH)_2$ of vitamin D (calcitriol - CT) depending on the form and period of the disease and to clarify the role of violations of the vitamin status of calcidiol and calcitriol in pathogenesis hemorrhagic fever with renal syndrome.

Patients and methods

114 patients with HFRS were under the supervision. The study was conducted in City Clinical Hospital No. 13 and Clinical Hospital for Infectious Diseases No. 4 in Ufa with a serologically confirmed diagnosis of HFRS. The patients were 94 men (82.4%), and 20 women (17.5%). The classification of Sirotin B. Z. was used in determining the severity of the disease [5]. The number of patients with moderately severe form was 53 people, with severe one - 61. The control group included 44 practically healthy individuals of the corresponding age (mainly doctors, teachers of schools and universities). The age of the examined was 18-55 years (average age 37.4 ± 2.6 years). The study excluded individuals who had diseases of the kidneys, liver, cardiovascular and nervous systems, as well as endocrinological ones preceding HFRS.

Blood for the study was taken from the ulnar vein in the amount of 9 ml after an overnight fast. The determination of 25 (OH) levels of vitamin D (calcidiol-KD) and 1.25 $(OH)_2$ of vitamin D (calcitriol-KT) in blood serum was carried out by high performance liquid chromatography (HPLC) in the Hemotest laboratory and in the ANO testing laboratory Center for Biotic Medicine.

The determination of the level of cytokines (tumor necrosis factor-alpha, gamma-interferon, interleukin-2, interleukin-4, interleukin-10) was carried out at the Central Research Laboratory of GBOU VPO BSMU of the Ministry of Health of Russia, Ufa. Reagent kits of Vector-Best CJSC (Novosibirsk) were used to determine the concentration of cytokines. Determination of the level of

cytokines was carried out by enzyme immunoassay in accordance with the manufacturer's instructions. The determination method is based on the “sandwich” variant of enzyme-linked immunosorbent assay using mono- and polyclonal antibodies.

Mathematical processing of the results of the study was carried out using the standard statistical software package Statistica 7.0 for Windows. The average value of M and the mean error of m were calculated in Microsoft Excel.

Study results and discussion

The study of serum vitamin levels was carried out depending on the severity of the disease, its phase and the main clinical syndrome activities. The obtained results of the content of vitamins are presented in table 1.

Our studies have shown that in the oliguric phase, the concentration of 1.25 $(OH)_2$ vitamin D - calcitriol (CT) in the blood serum is significantly reduced. Moreover, there is a marked deficiency of CT in the oliguric phase in patients with severe HFRS. In addition, the level of CT in this phase is significantly lower than in patients with moderately severe disease. The content of 25 (OH) vitamin D - caldiol (CD) in the blood serum of patients with HFRS below normal levels was detected only in the oliguric phase of severe HFRS. The concentration of CD as well as CT is significantly lower in severe HFRS than in the moderately severe course of the disease. The level of CD is within the normal range, and the level of CT only tends to normalize in the period of early convalescence.

Food Vitamin D is absorbed into micelles. It is transported in blood in connection to a specific transport globulin. In hepatocytes it is hydroxylated to 25-hydroxycholecalciferol (calcidiol or CD). This is the main reserve in the liver and transport in the blood form of vitamin D. Part of the CD is involved in the entero-hepatic circulation (like bile acids). If it is violated, vitamin D deficiency may occur. 25-hydroxycholecalciferol is hydroxylated at position 1 to form 1,25-dihydroxycholecalciferol (calcitriol or CT) in the kidneys, placenta, and

bones. Calcitriol production is regulated by its own concentration, parathyroid hormone and serum phosphate.

Calcitriol is the only regulator of calcium movement across the membrane of enterocytes against a concentration gradient. Calcitriol stimulates the biosynthesis of calcium-binding protein in enterocytes, which ensures the absorption of calcium and phosphates in the small intestine. CT enhances the reabsorption of calcium and phosphates in the renal tubules that helps to maintain their normal ratio in plasma and extracellular fluids. Thus, we revealed that the decrease in CT level respectively the severity of HFRS is one of the pathogenetic mechanisms of the hypocalcemia development in the HFRS oliguric phase.

In recent years, the active participation of vitamin D in the regulation of immunogenesis and cell proliferation has been shown. Monocytes and lymphocytes express the CT receptor protein with the same amino acid sequence as the intestinal CT receptor protein. Calcitriol directly affects CD 4+ T-lymphocytes, increasing the development of Th2 cells [6].

The study of serum cytokine levels was carried out depending on the severity of the disease, its phase and the main clinical syndrome activities. The obtained results of the content of cytokines are presented in table 2. Analysis of the content of the pro-inflammatory cytokine TNF-α during the febrile period revealed an increase in its concentration by 13.1 times in moderately severe form and by 18.6 times in severe form of HFRS higher than in the control group. A further increase in the level of TNF-α was noted both in the moderately severe form and in the severe one of HFRS in the oliguric phase. The TNF-α content decreased, remaining significantly higher than in the control group with moderately severe form and with severe one in the period of polyuria.

Analysis of the content of the pro-inflammatory cytokine IFN-γ revealed a completely different dynamics in contrast to TNF-α. The IFN-γ level was 6.94 ± 0.5 pg / ml, which is lower than the measurement of the control group ($p < 0.01$) in the period of polyuria. A further decrease in the level of IFN-γ was revealed

both in the moderately severe form and in the severe one of HFRS in the oliguric period. IFN-γ production increased slightly in moderately severe form and practically did not change in severe one in the phase of polyuria. The IFN-γ level was lower in convalescents of severe HFRS one month after discharge from the hospital than in the control group ($p < 0.05$). A decrease in the production of IFN-γ indicates a reduced activity of T-helpers and NK cells producing this cytokine. At the same time, low concentrations of IFN-γ can be explained by both its predominant content in tissues and rapid destruction. The lowest levels of IFN-γ were found in acute renal failure.

The level of IL-2, an important mediator of immunity, especially cellular in the febrile period of moderately severe form of HFRS did not differ from the control group, but decreased in severe form (5.4 ± 0.5 pg / ml, $p < 0.05$). A decrease in the level of IL-2 was revealed in the oliguric phase, $p < 0.05$. The concentration of IL-2 in patients with HFRS in the polyuric phase remained lower than in the control group, despite an increase in its level both with moderately severe form - 5.51 ± 0.5 pg / ml, $p < 0.05$, and with severe one - 4.14 ± 0.4 pg / ml, p0.05. By the convalescence period, the level of IL-2 did not differ from the control group in the moderately severe form of HFRS, but it was reduced in patients with severe one, $p < 0.5$. The main biological effect of IL-2 is to stimulate the proliferation of T and NK cells. Therefore, a low level of IL-2 is one of the factors that decrease the number of $CD3^+$and $CD16^+$ T-lymphocytes during the height of HFRS, which was previously identified.

Our studies showed the presence of a reliable direct correlation between calcitriol and IL-2 ($r = +0.61$ at $p < 0.05$), as well as between calcitriol and INF-γ ($r = +0.51$ at p0.05). In addition, a significant inverse correlation was found between the concentration of calcitriol and the level of proinflammatory cytokine TNF-α ($r = -0.53$ at $p < 0.05$).

According to scientific sources, activated T and B lymphocytes, macrophages and monocytes express special calcitriol receptors, which differentiate monocytes and prelimphocytes to mature forms, capable of

producing a sufficient amount of interleukins, growth factors, and other Ca-dependent immunogenesis mediators. Perhaps this can explain the direct correlation between CT and IL-2 that we identified, as well as between CT and INF-γ.

Thus, the lowest concentration of calcitriol and calcidiol in the blood serum is observed with moderately severe and severe forms of HFRS in the oliguric phase. The more severe the disease is, the more evident the deficiency of active vitamin D metabolites is. This fact may be one of the mechanisms contributing to the appearance of hypocalcemia affected hypocalceuria in the midst of HFRS.

Table 1

Average values of calcidiol and calcitriol in HFRS patients depending on the phase and severity of the disease (M ± m)

Phase	Severity form	25 (OH) Vitamin D (calcidiol - CD) ng/mL	1.25 $(OH)_2$ Vitamin D (calcitriol - CT) pg/ml
Control group		140.5 ± 12.4 * (normal range 8-320 ng / ml)	58.4± 2.4 * (normal range 16-65 pg / ml)
Febrile	Moderately severe	38.2 ± 3.1 *	32.2 ± 1.2 *
	Severe	26.3 ± 2.1 *	28.2 ± 1.1 *
	$p_{mod/sev}$	p <0.05	p <0.05
Oliguric	Moderately severe	9.4 ± 1.1 *	4.7 ± 0.3 *
	Severe	5.6 ± 1.1 *	2.6 ± 0.2 *
	$p_{mod/sev}$	p <0.05	p <0.05
Polyuric	Moderately severe	12.5 ± 1.2 *	11.2 ± 2.3 *
	Severe	9.1 ± 0.9 *	5.2 ± 1.1 *
	$p_{mod/sev}$	p <0.05	p <0.05
Reconvalescence	Moderately severe	19.6 ± 1.5 *	20.3 ± 1.2 *
	Severe	11.4 ± 1.2 *	16.1 ± 1.1 *
	$p_{mod/sev}$	p <0.05	p <0.05

* - reliability when compared to the same indicator in the control group, p <0.05.

The presence of correlations of calcitriol with IL-2, INF-γ, and TNF-α indicates a possible role of deficiency of the vitamin D active metabolite in the development of immunological disorders in HFRS.

Table 2

The content of circulating cytokines in patients with HFRS depending on the phase and severity of the disease (M ± m)

Phase	Severity form	INF - γ pg/ml	TNF-α pg/ml	IL-2 pg/ml
Control group		10.2 ± 1.15	2.4 ± 0.2	7.8 ± 0.71
Febrile	Moderately severe	8.5 ± 0.6	31.4 ± 2.5 *	7.1 ± 0.6
	Severe	6.94 ± 0.5 *	44.6 ± 3.2 *	5.4 ± 0.5
	$p_{mod/sev}$	p <0.05	p <0.05	p <0.05
Oliguric	Moderately severe	5.87 ± 0.5 *	69.6 ± 5.3 *	4.23 ± 0.4 *
	Severe	4.42 ± 0.4 *	89.4 ± 6.2 *	3.07 ± 0.3 *
	$p_{mod/sev}$	p <0.05	p <0.05	p <0.05
Polyuric	Moderately severe	6.92 ± 0.6 *	45.3 ± 4.1 *	5.51 ± 0.5 *
	Severe	4.52 ± 0.3 *	61.2 ± 5.2 *	4.14 ± 0.4 *
	$p_{mod/sev}$	p <0.05	p <0.05	p <0.05
Reconvalescence	Moderately severe	7.85 ± 0.6	28.8 ± 1.1 *	6.2 ± 0.5
	Severe	5.24 ± 0.4 *	39.3 ± 3.2 *	5.24 ± 0.4 *
	$p_{mod/sev}$	p <0.05	p <0.05	p > 0.05

* - reliability when compared to the same indicator in the control group, p <0.05.

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REFERENCES

1. Khasanova G.M., Tutelyan A.V., Valishin D.A. Dynamics of cytokine values in patients with hemorrhagic fever with renal syndrome. // Infectious diseases. - 2011. - T. 9. №3. - P. 31-34.

2. Khasanova G.M., Tutelyan A.V., Valishin D.A. Relationship balance vitamins, microelements and cytokine status haemorrhagic fever with

renal syndrome // Russian journal of immunology. - 2013. - Vol. 7 (16). №4. - P. 445-450.

3. Khasanova G.M. Relationship of the level of circulating cytokines and microelements in patients with hemorrhagic fever with renal // Saratov Journal of Medical Scientific Research - 2011. - T. 7. №4. - P. 863-865.

4. Zharskiy S.L. State of calcium and phosphorus exchange in acute renal failure in patients with hemorrhagic fever with renal syndrome. Abstract. diss. … candidate of medical sciences. - Khabarovsk, - 1991. - 24 p.

5. Sirotin B.Z. Hemorrhagic fever with renal syndrome - Khabarovsk. - 1994. – 302 p.

6. Rusnak F.I. Vitamin D and the progression of kidney disease. Scientific and technical development bulletin. – 2009. - № 11 (27). - P. 52-64.