

**EVALUATION OF INTERFERON- $\alpha$  PRODUCTION POTENTIAL OF IMMUNE CELLS AND THE LEVEL OF CRYOGLOBULINS IN PATIENTS WITH CHRONIC VIRAL HEPATITIS B AND C IN UZBEK POPULATION.**

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**ABSTRACT**

Interferon- $\alpha$  (IFN- $\alpha$ ) is the key cytokine in defense against viruses. Major IFN- $\alpha$  producer is plasmacytoid dendritic cells, which reside mainly in blood. Spontaneous and mitogen-induced productions are very informative characteristics of immune cells potential, which play crucial role in clearance of viruses. Nowadays, viral hepatitis imposes heavy burden on many countries healthcare, and countries of Central Asia aren't exception. In order to study regional particularities of viral hepatitis in Uzbekistan, our laboratory has studied spontaneous and mitogen-induced production of IFN- $\alpha$  in patients with chronic viral hepatitis B and C (CVHC and CVHB) of Uzbek population. **Conclusion:** In patients with CVHC and CVHB spontaneous production of IFN- $\alpha$  didn't differ significantly in comparison with results of control group. Whereas mitogen-induced production of IFN- $\alpha$  was nearly twice less than in control group.

**KEYWORDS:** Spontaneous, mitogen-induced, production of intereron- $\alpha$ , cryoglobulins, hepatitis B and C.

**INTRODUCTION**

Interferon- $\alpha$  is a pleiotropic cytokine, with the prevalence of antiviral effect<sup>[1]</sup>, which makes it the first protective line against viruses. The mechanism of the antiviral effect of IFN- $\alpha$  has been studied quite thoroughly.<sup>[2]</sup> IFN- $\alpha$  drugs are used to treat many viral diseases, including viral hepatitis B and C, and is the gold standard of treatment for these diseases. But, despite this, the effectiveness of treatment leaves much to be desired and many patients remain untreated.<sup>[3] [4]</sup> According to WHO, in 2015, about 325 million people were infected with viral hepatitis B and C and due to 1.34 million people (about 2.5 people per minute) died. In view of the foregoing, one can easily feel a sharp need for studying the pathogenesis of viral hepatitis. Since IFN- $\alpha$  is of great importance in the pathogenesis of viral hepatitis and the study of spontaneous and mitogen-induced products provides valuable information on the functional state of immune cells. Spontaneous and mitogen-induced levels of IFN- $\alpha$  in patients CVHC and CVHB were studied by our laboratory. In addition, the level of cryoglobulins was assessed, which is often observed in viral hepatitis, especially in viral hepatitis C. It is known that spontaneous production of cytokines is the production of a specific cytokine by peripheral blood cells in vitro, which indicates the in vivo activation of

immune cells in the context of infection and physiological processes and was more frequent in regions where infections were endemic.<sup>[5]</sup> Mitogen-induced production of cytokines is the synthesis of cytokines in vitro with leukocytes activated by mitogen<sup>[6]</sup>, which indicates the preserved potential of immune cells.

Cryoglobulins are immunoglobulins that precipitate at a temperature below 37°C and dissolve upon reheating.<sup>[7]</sup> Cryoglobulins are found in various pathologies including CVHB and CVHC, most often cryoglobulins are detected in patients with viral hepatitis C, and this disease accounts for about 73% of the cases of cryoglobulins detected.<sup>[8]</sup> The authors claim that of the 2323 patients with viral hepatitis C, 44% had cryoglobulins. Cirrhosis of the liver was established in 40% of patients with cryoglobulins, which indicates a strong relationship of cryoglobulins and liver cirrhosis<sup>[9]</sup> and emphasizes the diagnostic significance of cryoglobulins.

**MATERIAL AND METHODS**

We sampled blood from 29 patients (including 12 men and 17 women) with chronic viral hepatitis (8 CVHB and 21 CVHC) in the department of chronic hepatitis of

Clinic of Uzbekistan scientific research institution of epidemiology, microbiology and infectious diseases, the Ministry of Healthcare of the Republic of Uzbekistan. Diagnosis was established on the basis of clinical and laboratory studies in the hospital. Clinically, chronic hepatitis manifested by: rapid fatigue (in 41% of patients), weakness (in 62% of patients), decreased appetite (in 65% of patients), pain in the right upper quadrant (in 72% of patients), flatulence (in 48% of patients), nausea (in 34% of patients), bitter taste in mouth (in 24% of patients), headaches (in 17% of patients). The laboratory parameters in patients were as follows: Bilirubin  $25.8 \pm 2.9 \mu\text{m} / \text{L}$ , Alanine transaminase (ALT)  $0.01 \pm 0.05 \text{ mmol} / \text{l}$ , aspartate aminotransferase (AST)  $0.049 \pm 0.02 \text{ mmol} / \text{l}$ . The patients' blood was taken to a sterile tube, centrifuged for serum collection, and the level of IFN- $\alpha$  was determined (as in the other cases) using Vector-Best ELISA kits (Novosibirsk, Russia). To determine the spontaneous and mitogen-induced production of IFN- $\alpha$ , patients' blood was collected into 2 sterile tubes with an anticoagulant. The nutrient medium 199 (Hank's solution) was added to the first tube and left for 24 hours in a thermostat at  $+37^\circ\text{C}$ . Hank's solution and virus particles (which were used as mitogen for IFN- $\alpha$  production) were added into second tube and left for 24 hours at  $+37^\circ\text{C}$ . After 24 hours, tubes with blood were centrifuged, the supernatant was collected and the level of IFN- $\alpha$  was tested therein. In parallel, the above manipulations were carried out using the blood of practically healthy individuals, who made up the control group (Table 1). Cryoglobulins have been studied using the method for determining circulating immune complexes (CIC) [Haskovo et al.]. CIC were measured by optical density (OD) in 96-well plate using spectrophotometer at 450 nm wave-length and as reference filter at 630 nm wave-length. Large (IgM-containing) and small (non-IgM-containing) CIC were measured after 24 hours of incubation at  $+4^\circ\text{C}$ . Next, the test sera were placed in a thermostat, incubated at  $+37^\circ\text{C}$  for 1 hour, and the CIC was measured again. The difference in the level of the CIC between the two incubations was taken as the level of cryoglobulins (incubation  $+4^\circ\text{C}$  - incubation  $+37^\circ\text{C}$ ). As a control, the indices of cryoglobulins of practically healthy people were taken (large CIC  $0.0112 \pm 0.017 \text{ OD}$ , small CIC  $0.0122 \pm 0.015 \text{ OD}$ ). The obtained data were statistically

processed using the Microsoft Excel 2013 program, calculating the arithmetic mean (M), the standard deviation ( $\sigma$ ), the standard error (m), the Student's criterion (t) and calculating the error probability (P).

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## RESULTS AND DISCUSSION

Data obtained on mitogen-induced, spontaneous and serum levels of IFN- $\alpha$  production in patients with CVHC and CVHB are presented in the Table 1. Analysis of the results showed that the synthesis potential of IFN- $\alpha$  tended to decrease more than 2-fold in the groups of patients with chronic viral hepatitis relative to the control group. In the control group, the mitogen-induced production of IFN- $\alpha$  was  $112.9 \pm 38.2 \text{ pg} / \text{ml}$ , while in the group of patients with CVHC and CVHB this index was  $46.5, 3 \pm 7.3 \text{ pg} / \text{ml}$  ( $p > 0.05$ ) and  $45, 9 \pm 12.3 \text{ pg} / \text{ml}$  ( $p > 0.05$ ), respectively. Therefore, it can be concluded that in patients with chronic viral hepatitis, as expected, the potential of IFN- $\alpha$  production is relatively diminished, since a chronic viral infection in the body of patients leads to a exhaustion of immune cells<sup>[10]</sup>, particularly, plasmacytoid dendritic cells, which are the main producers of IFN- $\alpha$ .<sup>[11]</sup> Concerning the spontaneous production of IFN- $\alpha$ , only in CVHC group, marginal spontaneous production was observed. While in other groups the spontaneous production of IFN- $\alpha$  was lower than that of the serum IFN- $\alpha$ . It should also be noted that there is no clear difference in the level of spontaneous IFN- $\alpha$  production between the groups: in the control group  $14.7 \pm 3.5 \text{ pg} / \text{ml}$ ; in patients with CVHC disease  $14, 1 \pm 0, 8 \text{ pg} / \text{ml}$  ( $p > 0, 05$ ); in patients with CVHB  $14, 2 \pm 1, 2 \text{ pg} / \text{ml}$  ( $p > 0.05$ ). These results, presumably, can be explained by the absence of inflammation in healthy individuals and by the decreasing of the threshold for activation of peripheral blood leukocytes in patients. Serum IFN- $\alpha$  differed insignificantly: in healthy subjects it was  $17.85 \pm 4.7 \text{ pg} / \text{ml}$ ; in patients with CVHC -  $12.7 \pm 0.3 \text{ pg} / \text{ml}$  ( $p > 0.05$ ); in patients with CVHB -  $15.7 \pm 2.1 \text{ pg} / \text{ml}$  ( $p > 0.05$ ).

**Table 1. IFN- $\alpha$  in patients with CVHC and CVHB.**

	Control	CVHC	CVHB
Serum pg / ml	17,85±4,7	12,7±0,3	15,7±2,1
Spontaneous production pg / ml	14,7±3,5	14,1±0,8	14,2±1,2
Mitogen-induced production pg / ml	112,9 ± 38,2	46,5±7,3	45,9±12,3

In addition, the stimulation index (the ratio of mitogen-induced to spontaneous production) in the group of patients with CVH and in the control group was calculated. In the control group, the stimulation index was  $8.9 \pm 2.8$ , whereas in patients with CVH this index was  $3.5 \pm 0.6$ , which is several times less than in the control group. The obtained results confirm the above-

described hypothesis of the exhaustion of immune cells by prolonged viral load in patients with CVH. Concerning cryoglobulins, in the group of CVH they were  $0.023 \pm 0.044 \text{ OD}$  (large CICs) and  $0.036 \pm 0.077 \text{ OD}$  (small CICs), which is several times higher than the control group (large CIC  $0.0112 \pm 0.017 \text{ OD}$ , small CIC  $0.0122 \pm 0.015 \text{ OD}$ ).

## CONCLUSIONS

In patients with CVHC and CVHB, the mitogen-induced level of IFN- $\alpha$  production was significantly lower than in the control group, which gives grounds for the assumption that IFN- $\alpha$  synthesis in peripheral blood leukocytes in patients with chronic viral hepatitis is exhausted after prolonged activation by viruses. Similar results were obtained by other authors earlier.<sup>[11]</sup>

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