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Evaluation of Indicators of the Immune System in Patients Suffering from Chronic Viral Hepatitis B

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Abstract

The analysis of indicators of nonspecific and specific resistance in patients with chronic viral hepatitis B was carried out. Relative indicators of CD3 and CD19 of regular subpopulations of the T-link of immunity (CD4 and CD8), N-lymphocytes, immunoregulatory index (CD4/CD8) are presented. After treatment with bificol against the background of traditional therapy in patients with chronic viral hepatitis B, it was noted that all indicators of cellular and humoral immunity, as well as parameters of nonspecific resistance, were significantly higher than in patients who did not receive the probiotic.

Keywords: chronic viral hepatitis, immunity, protective factors, eubiotics

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Оценка показателей системы иммунитета у пациентов, страдающих хроническим вирусным гепатитом В

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Резюме

Проведен анализ показателей неспецифической и специфической резистентности у пациентов с хроническим вирусным гепатитом В. Представлены относительные показатели CD3 и CD19 регулярных субпопуляций Т-звена иммунитета (CD4 и CD8), N-лимфоцитов, иммунорегуляторного индекса (CD4/CD8). После лечения бификолом на фоне традиционной терапии у пациентов с хроническим вирусным гепатитом В было отмечено, что все показатели клеточного и гуморального иммунитета, а также параметры неспецифической резистентности были достоверно выше, чем у пациентов, не получавших пробиотик.

Ключевые слова: хронический вирусный гепатит, иммунитет, факторы защиты, эубиотики

■ INTRODUCTION

It is known that the normal flora of human cavities stimulates the lymphoid apparatus, the synthesis of immunoglobulins, increases the activity of lysozyme, increases the level of the secretory fraction of class A immunoglobulin, as well as the phagocytic activity of neutrophils [1–3].

One of the main achievements of modern medicine as a biological science is the rapid development of immunology. Having first appeared in the depths of infectology, it created the necessary basis for the formation of a fundamental discipline that studies the regulation of not only immunological homeostasis, but homeostasis in general. Close connections of the immune system with the nervous, endocrine and other systems, participation in the process of fertilization and pregnancy, various types of metabolism, in the regulation of the life expectancy of an individual, etc., were revealed. In the last few decades, the fact of the development of immune disorders has been established, which was expressed both in a decrease and in an increase in the values of immune parameters

from the level of the functional norm, which is the cause or consequence of various pathological reactions, but in any case, a factor in its aggravation, chronicity.

■ MATERIALS AND METHODS

To study the immune status, we selected 37 patients with viral hepatitis B, from whom 5–10 ml of blood was taken with a sterile syringe. in a ratio of 1:2, carefully layered on a separating solution of phenolverographin ($p=1.077$) and centrifuged for 30 min at 1500 rpm at room temperature. Lymphocytes accumulated in the form of a white layer at the interface between the separating solution and the medium were collected with a pipette and washed with medium 199 at 1000 rpm at room temperature for 5 min. The precipitate consisting of lymphocytes was diluted with Hanks' medium to a concentration of 2×10^6 ml.

The phenotype of immunocompetent cells was determined using monoclonal antibodies (manufactured by the Institute of Immunology of the Russian Federation) to the differentiation markers CD3 (total pool of T-lymphocytes), CD4 (T-helpers), CD8 (cytotoxic suppressors), CD19 (B-lymphocytes).

The viability of lymphocytes isolated from peripheral blood was determined by the method of B.V. Brondz. To do this, 1.9 ml of 0.1% eosin solution (dissolved in distilled water) and 1.9 ml of 0.15% trypan blue (4x Ringer's solution) were added to 0.1 ml of lymphocyte suspension. Dead cells were stained blue. By microscopy in a thick drop under a coverslip, the percentage of dead cells was calculated.

Populations and subpopulations of lymphocytes were determined using monoclonal antibodies. To determine the immunoglobulins of class M, A, G, the method of radial immunodiffusion according to Mancini was used.

The phagocytic index of neutrophils was determined using freshly isolated cultures (18 hours) of staphylococci from latex.

To determine the titer of lysozyme in the blood serum or in the oral fluid, the agar diffusion method was used using sterile paper disks according to the method proposed by Sh.R. Aliyev and D.A. Umarova.

Statistical processing of the research materials was carried out using a package of standard statistical programs Excel with the inclusion of the analysis of descriptive variation statistics, as well as correlation analysis.

■ RESULTS

Currently, there are numerous data on the dependence of the clinical course of chronic viral hepatitis B on the functional state of the immune system [4, 5]. This section presents the results of studying the indicators of cellular and humoral immunity, as well as the state of nonspecific resistance in patients with chronic viral hepatitis B. Table 1 shows the relative indicators of CD3 and CD19 of regular subpopulations of the T-link of immunity (CD4 and CD8), N-lymphocytes, immunoregulatory index (CD4/CD8).

Examination conducted in patients with chronic viral hepatitis B, upon admission, revealed multidirectional changes in immune homeostasis. So, to a greater extent, these changes relate to nonspecific resistance and indicators of cellular immunity, while indicators of humoral immunity concern to a lesser extent.

Thus, in all patients with chronic viral hepatitis B, there was a significant decrease in relative CD3 values to $48.5 \pm 2.15\%$ versus $61.2 \pm 1.41\%$ in the control group. Against the

Table 1
Dynamics of indicators of the immune system in patients with chronic viral hepatitis B after traditional treatment

№	Indicators	Control (n=20)	In patients	
			On admission (n=23)	After traditional treatment (n=7)
1	CD3, %	61.2±1.41	48.5±2.15	44.6±2.71
2	CD4, %	32.0±0.71	27.0±1.7	24.4±2.6
3	CD8, %	18.0±0.51	12.0±0.75	12.3±1.32
4	CD19, %	19.1±0.81	23.0±1.15	21.5±1.41
5	N lymphocytes, %	17.3±0.32	26.2±1.61	36.1±4.61
6	Ig A, g/l	1.6±0.04	1.8±0.07	2.0±0.12
7	Ig M, g/l	0.90±0.04	0.5±0.04	0.81±0.03
8	Ig G, g/l	12.1±0.32	9.30±0.41	11.4±0.61
9	FAN, %	56.2±1.51	46.0±1.62	44.0±3.11
10	Active Lysozyme	26.3±0.81	12.0±0.62	16.3±1.21
11	CD4/CD8	1.7±0.10	2.12±0.1	1.95±0.3

Note: n is the number of examined patients.

background of traditional treatment, CD3 counts continue to decrease to 44.6±2.71%, but the differences were not significant. In general, these figures remained reduced, despite the severity of the clinical picture of the disease. It should be noted that there is a significant decrease in CD8 values to 12.0±0.75% at a rate of 18.0±0.51%. CD4 counts were also reduced after traditional therapy by 1.2 times. At the same time, in general, there was a tendency to increase the studied indicators, but they did not reach the control values.

When studying humoral immunity, the following changes were revealed in the indicators: the level of CD19 increased to 23.0±1.15%; however, after traditional therapy, this figure decreased to 21.5±1.41%. The concentration of IgA also tended to increase, especially after traditional therapy, and amounted to 2.0±0.12%. At the same time, there was a decrease in the content of IgM and IgG, which amounted to 0.81±0.03% in the control was 0.90±0.04%, and 9.30±0.41 in the control 11.4±0.61%. In addition, there is a decrease in non-specific protective factors that cause the development of inflammatory processes in various organs and tissues.

Consequently, in patients with chronic viral hepatitis B, dysimmunoglobulinemia is noted, which is characterized by an increase in the level of IgA and a decrease in IgM and IgG. The traditional therapy administered to these patients had no effect on the immunological reactivity of the body. At the same time, the study of indicators of nonspecific protection factors in these same patients revealed a decrease in the phagocytic activity of neutrophils to 46.0±1.62% and to 44.0±3.11% after treatment. The activity of lysozyme was also reduced and amounted to 12.0±0.62% before treatment, and after treatment it was 16.3±1.21%, while in the control group it was 26.3±0.81%.

The immunoregulatory index is 2.12±0.1%. Based on the notion that CD4s are able to enhance the body's immune response to antigenic irritation, and CD8s provide regulation of the duration of the intensity of the immune response, the revealed imbalance of T-cell subpopulations explains to some extent the defect in immune regulation in patients with

chronic viral hepatitis B, possibly associated with the severity the course of the disease. In other words, the detected decrease in CD4 proves the weakening of the body's defense response.

Thus, we can conclude that traditional therapy of patients does not lead to normalization of immunity parameters. Apparently, this is due to the development of secondary immunodeficiency against the background of microbial overgrowth syndrome.

Taking into account the above factors, which reliably indicate that traditional treatment for patients with chronic viral hepatitis B does not lead to reliable positive results both in terms of flora and immune parameters, we for the first time in Uzbekistan treated patients using the eubiotic bifcol in addition to traditional treatment.

Eubiotic bifcol is a combined preparation developed on the basis of a local (RU) strain of *Bifidum longum* and *Escherichia coli*. According to the creators of the drug [6], microbial strains of local origin may have advantages over foreign ones due to the greater similarity of their receptor apparatus with intestinal epitheliocytes of the local population [7]. According to available data, the drug is rich in complete amino acids, fats, vitamins, includes highly sensitive cells that restore disturbed microflora and inhibit the growth of pathogenic microorganisms due to the presence of antagonistic properties.

■ DISCUSSION

After treatment with bifcol against the background of traditional therapy in patients with chronic viral hepatitis B, it was noted that all indicators of cellular and humoral immunity, as well as parameters of nonspecific resistance, were significantly higher than in patients who did not receive a probiotic (Table 2). Thus, the number of CD3 in patients significantly increased. The number of CD4 and CD8 also increased, although not significantly. At the same time, a decrease in the content of B-lymphocytes to $20.6 \pm 1.7\%$ was noted.

The study of indicators of humoral protection factors in the blood serum of patients showed that the content of IgM significantly decreased and amounted

Table 2
Parameters of the immune system in patients with chronic viral hepatitis B treated with bifcol

№	Indicators	Control (n=20)	In patients	
			On admission (n=23)	After traditional treatment (n=7)
1	CD3, %	62.3±1.2	42.1±1.8	50.0±2.3
2	CD4, %	33.0±0.6	25.2±1.3	29.0±1.15
3	CD8, %	18.0±0.5	13.5±0.5	15.0±0.8
4	CD19, %	19.2±0.7	23.2±0.6	20.6±1.7
5	N lymphocytes, %	18.6±0.3	31.3±2.0	25.0±1.5
6	Ig A, g/l	1.6±0.05	1.8±0.09	1.7±0.12
7	Ig M, g/l	0.7±0.04	13.0±0.02	1.1±0.06
8	Ig G, g/l	10.8±0.35	10.4±0.31	10.2±0.6
9	FAN, %	55.0±1.5	44.0±1.3	49.0±2.3
10	Active Lysozyme	27.0±0.8	10.7±0.7	15.6±1.1
11	CD4/CD8	1.7±0.10	2.5±0.4	2.0±0.2

Note: n is the number of examined patients.

to $1.10 \pm 0.06\%$ g/l, while the level of IgG did not change and amounted to 10.26 ± 0.6 g/l. The index of phagocytic activity of neutrophils at admission was reduced, and increased during treatment with a probiotic, although it did not reach the control level. At the same time, the titer of lysozyme was significantly higher up to $15.6 \pm 1.1\%$ mg than in patients who did not take the probiotic.

Thus, the analysis of the immunological parameters performed in patients with chronic viral hepatitis B who took the probiotic bificol showed a more pronounced increase in the level of T- and B-lymphocytes than in patients who received only traditional therapy. Thus, it can be noted that in the group who used the probiotic, the content of CD3 increased to $50.0 \pm 2.3\%$, CD4 – up to $29.0 \pm 1.15\%$, CD8 – up to $15.0 \pm 0.8\%$, but did not reach the control.

■ CONCLUSIONS

Considering the analysis of all immunological studies of blood serum in patients with chronic viral hepatitis B, it is possible to draw the following conclusions:

1. In patients with chronic viral hepatitis B, there is a pronounced secondary immunodeficiency in terms of cellular, humoral immunity and nonspecific protective factors.
2. In patients with chronic hepatitis B who received traditional therapy, there are no significant positive changes in all indicators of the immune system.
3. In patients with CVHB who received, along with traditional therapy, the probiotic bificol, there were significant, positive changes in the parameters of the immune systems in all studied parameters. In fact, these changes are very close to the benchmarks.

■ REFERENCES

1. Dagushina V.F. Study of the immunocorrective properties of bacterial preparations. *Journal of Microbiology*. 1991;4:56–58. (in Russian)
2. Mukhamedov I.M. *Dentistry and clinics microbiology*. T. Monograph. 2016; 600 p. (in Russian)
3. Mukhamedov B.I. Violations of microecology and local factors of protection of the oral cavity in patients with viral hepatitis B. *Medicine and innovations*. 2022;4(8):345–353. (in Russian)
4. Khaitov R.M. *Immunology*. M., 2010; 400 p. (in Russian)
5. Petrov R.V. Immunodiagnosics of immunodeficiencies. *Immunology*. 1997;4:4–9. (in Russian)
6. Ogay D.K., Elova N.A. Study of sensitivity to antibiotics of lactic acid bacteria and bifidobacteria. *Proceedings of the Institute of Microbiology of the National Academy of Sciences of Azerbaijan*. 2013;11(1):376–380. (in Russian)
7. Mukhamedov I.M. *Clinical microbiology*. Tashkent, 2018; 600 p. (in Russian)