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L.Yu. Ilchenko*[1,2,3], I.A. Morozov[1], T.V. Kozhanova[1], N.V. Soboleva[1], l.I. Melnikova[3], I.V. Kruglova[4]

[1] — Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of Russian Academy of Sciences, Moscow, Russia

[2] — Pirogov Russian National Research Medical University, Moscow, Russia

[3] — Clinical hospital № 85 FMBA of Russia, Moscow, Russia

[4] — Federal Scientific and Clinical Center for Sports Medicine and Rehabilitation FMBA Russia, Moscow Russia

The Frequency of Hepatitis Virus Infection Markers Among Highly Qualified Sportsmen

Abstract

Study Objective is to evaluate prevalence of hepatitis A, B, C, E, and TT virus infection markers in highly qualified sportsmen. **Study Design:** multicenter open single-site clinical study. **Materials and Methods:** 100 blood serum samples of sportsmen (game, complex coordination, technical, etc.) were studied. Biological material (blood serum) was obtained from 54 men and 46 women aged 16 to 45 years during an in-depth medical examination. All sportsmen filled out a questionnaire, including demographic data, description of the sport, information about infection risk factors, information about the presence of acute viral hepatitis and vaccination. Anti-HAV IgG, HBsAg, anti-HBcore, anti-HCV, anti-HEV IgG, anti-HEV IgM were determined in the blood serum by enzyme immunoassay; using polymerase chain reaction — DNA Anelloviridae (TTV, TTMDV, TTMV). **Study Results:** Anti-HAV IgG was detected in 57/66 (86,4%) sportsmen, women (91,2%) predominated, among them a third were engaged in synchronized swimming. 7/57 (12,3%) of the examined had indications of vaccination against hepatitis A. The frequency of anti-HEV IgG did not exceed 3% (2/66). anti-HEV IgM were not detected in any case. Also, none of the examined sportsmen in the blood serum was not determined HBsAg. However, anti-HBcore (marker of latent HBV infection) was detected in 13% (13/100) of the samples. The detection rate of anti-HCV was low, combined with the presence of anti-HBcore was 2% (2/100). In addition, DNA TTV, TTMDV and TTMV, respectively, were found in serum samples from 66/100 (86%), 79/100 (79%), 71/100 of sportsmen. **Conclusion:** The high frequency of hepatitis virus markers was found (HAV — 74,1%, TTV/TTMDV /TTMV — 71-86%), HBV — 13%, HEV — 3%, HCV — 2%). All patients denied a history of acute viral hepatitis. Vaccination against hepatitis A and B is a modern strategy that prevents infection and the development of acute viral hepatitis. Its mandatory holding should become part of the targeted preparation of sportsmen to achieve the highest sports results.

Key word: *highly qualified sportsmen, markers of hepatitis viruses A, B, C, E, TT*

Conflict of interests

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*Contacts: Lyudmila Yu. Ilchenko, e-mail: ilchenko-med@yandex.ru

ORCID ID: https://orcid.org/0000-0001-6029-1864

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AHB — acute hepatitis B, AHC — acute hepatitis C, anti-HAV IgG — antibodies to hepatitis A virus, class G immunoglobulins, anti-HBcore IgG/IgM — antibodies to hepatitis B virus capsid antigen, class G/M immunoglobulins, anti-HCV — antibodies to hepatitis C virus, anti-HEV IgG/IgM — antibodies to hepatitis E virus, class G/M immunoglobulins, HA — hepatitis A, HB — hepatitis B, bp — base pair, CHB — chronic hepatitis B, CHC — chronic hepatitis C, DME — detailed medical examinations, FMBA — Federal Medical and Biological Agency, HBsAg — hepatitis B surface antigen, HBV DNA — hepatitis B deoxyribonucleic acid, HC — hepatitis C, HCV RNA — hepatitis C ribonucleic acid, HE — hepatitis E, PCR — polymerase chain reaction, TTMV — Torque teno mini virus, TTMDV — Torque teno midi virus, TTV — Torque teno virus

Introduction

Physical training is a means of preventing hypodynamia and promoting health. It has a many-sided effect on the human body and increases the body's resistance to adverse ambient effects. Sport is one part of physical training, and high-performance sport is an activity aimed at achieving high results. It requires the mobilization of the emotional state and all functional capabilities of the body.

Achieving high sports results is based on the research and methodological support of athletes' training system during different periods of the training and competitive process. Biomedical support is the study of the competitive activity of athletes, as well as comprehensive, routine and detailed medical examinations (DME) that are carried out according to custom-tailored programs developed at the medical institutions of the Federal Biomedical Agency (FMBA) of Russia [1].

Medical monitoring is performed to assess the health, biological age, physical fitness, level of training, and special abilities of the athlete, and identify overstrain. It includes morphophysiological, ergometric, biochemical, psychophysiological, and sports and pedagogical study methods [2].

The hepatobiliary system is one of the most critical systems that provide an adequate response of the body to intense physical activity. Maladaptation of the hepatobiliary system results in different types of pain in the right hypochondrium at rest and during physical exertion. It is also often accompanied by dyspeptic symptoms (bitter or metallic taste in the mouth, heartburn, intolerance to fatty and fried foods). On palpation, there is tenderness in the liver and gall bladder area; the liver may be enlarged. Signs of liver maladaptation and overstrain include increased activity of aminotransferases (1.5–2 times higher than the upper limit of normal).

Clinical and laboratory methods, ultrasound of the abdomen, test breakfasts, and test loads are used to identify hepatobiliary disorders.

In the last few years, the number of athletes with hepatobiliary diseases has risen due to increased physical activity, nutrition problems, uncontrolled use of pharmacological agents, etc.

However, among etiological factors leading to hepatic diseases, hepatitis viruses remain the most significant. According to the State Report "On the sanitary and epidemiological well-being of the population in the Russian Federation in 2018", the incidence of acute hepatitis B (AHB) per 100 thousand people in 2018 was 0.67; that of chronic hepatitis B (CHB) — 9.27; acute hepatitis C (AHC) — 1.1; chronic hepatitis C (CHC) — 32.72; hepatitis A (HA) — 2.84; hepatitis E (HE) — 0.11 [3]. Infections caused by the hepatitis delta virus and Torque teno virus (TTV) are not officially recorded in our country.

Viral hepatitis can be asymptomatic for a long time and, therefore, can escape detection and control. According to epidemiologists, the prevalence of viral hepatitis is associated with the deterioration of living conditions, which contributes to infection routes.

It should be noted that the epidemic process of viral hepatitis depends on the level of herd immunity, and its main characteristic is the prevalence of infection markers in the population. Therefore, only the screening of representative populations yields objective data on the viral load in the population. The optimal strategy for this kind of assays is the detection of markers in blood serum [4].

The objective of our study was to assess the detection rate of serological markers of hepatitis A (HAV), B (HBV), C (HCV), E (HEV), TT in professional athletes.

Materials and Methods

A multicenter, open, cross-sectional clinical trial was conducted at Clinic No. 5 of the Federal Clinical Hospital No. 85 of the Federal Medical and Biological Agency (Federal State Budgetary Healthcare Institution Clinical Hospital No. 85 of the Federal Medical and Biological Agency, Center for Diagnosis and Management of Chronic Viral Hepatitis) and the Federal Clinical Center for Sports Medicine and Rehabilitation of the Federal Medical and Biological Agency (Federal State Budgetary Institution Federal Clinical Center for Sports Medicine and Rehabilitation of the Federal Medical and Biological Agency of Russia). The clinical trial protocol (No. 157 of September 19, 2018) was approved by the Local Ethics Committee of Clinical Hospital No. 85 of the Federal Medical and Biological Agency of Russia.

During DME, blood samples were taken from 340 professional athletes aged 16 to 45 years. All individuals enrolled in this study signed informed consent for blood sampling, test for hepatitis virus markers, and the publishing of the results.

The athletes also filled a questionnaire drawn up by the researchers.

Blood samples were transported to the Chumakov Federal Scientific Center for Research and Development of Immunobiological Products of the RAS (Federal State Budgetary Institution Chumakov Federal Scientific Center for Research and Development of Immunobiological Products of the Russian Academy of Sciences) where hepatitis virus markers were determined in the Laboratory for the Simulation of Immunobiological Processes with experimental models on marmoset monkeys.

Serological markers of HAV, HEV, HBV and HCV (anti-HAV Ig, anti-HEV IgG/IgM, HBsAg, anti-HBcore, anti-HCV) were detected by enzyme immunoassay using the following test systems in accordance with manufacturer instructions: Monolisa Total Anti-HAV (Bio-Rad, France); DS-EIA-ANTI-HEV-G; DS-EIA-ANTI-HEV-M; DS-EIA-HBsAg-0.01; DS-EIA-HBsAg-0.01-confirming; DS-EIA-HBs; DS-EIA-HCV, DS-EIA-ANTI-HCV-SPECTR-GM (Diagnostic Systems Research and Manufacturing Association, Nizhny Novgorod). Sixty-six out of 100 athletes were tested for anti-HAV Ig and anti-HEV IgG/IgM.

All blood serum samples were tested for deoxyribonucleic acid (DNA) of the viruses of the Anneloviridae family using polymerase chain reaction (PCR). Nucleic acids were isolated from serum samples using "Kit for the isolation of deoxyribonucleic acid / ribonucleic acid (DNA/RNA) from serum or plasma using MP@SiO_2 magnetic particles" — the reagent kit manufactured by ZAO Sileks, according to the manufacturer's instructions.

Blood serum samples were analyzed using PCR with nested primers proposed by M. Ninomiya et al. [5] to detect DNA of the viruses of the Anneloviridae family. This allowed differentiating TTV, Torque teno midi virus (TTMDV) and Torque teno mini virus (TTMV) based on the size of the amplified fragment. The amplification products had the following sizes: 112–117 nt for TTV, 88 nt for TTMDV, and 70–72 nt for TTMV. The obtained product with 207 base pairs (bp) was determined by electrophoresis in 2% agarose gel in TBE buffer (Tris-borate-EDTA).

Questionnaire of a highly qualified athlete

Full name	
Age	
Gender	Male / Female
Birthplace	
Type of sport	
Sports qualification	
A history of acute viral hepatitis	A, B, C, E (when?)
HAV vaccination	yes, no (when?)
HBV vaccination	yes, no (when?)
Surgery	yes, no (when?)
Blood transfusions	yes, no (when?)
Dental care	yes, no (when?)
Tattoos, piercings	yes, no (when?)
Acupuncture	yes, no (when?)
Departure to foreign countries (indicate which)	
Contact with patients with viral hepatitis	
Blood sampling date	

Statistical data analysis was performed using EXCEL 2010 and GraphPad Prism 4 statistical data processing software. Data are presented as M ± SD, where M is the arithmetic mean, and SD is the standard deviation.

Results and Discussion

This paper analyzes preliminary findings. The study sample of athletes whose blood samples were included in the study (100 out of 340) was formed by random sampling. The athletes were involved in various sports (team, technical, with precise coordinated movements, etc.) (Table 1).

The questionnaire survey enabled to obtain a sociodemographic description of athletes and data on risk factors for hepatitis viruses, as well as information on the history of acute viral hepatitis (A, B, C, E) and vaccination against hepatitis A (HA) and hepatitis B (HB). Among the respondents, 35% (35/100) of athletes were Candidates for Master of Sports, 39% (39/100) — Masters of Sports, 14% (14/100%) — Masters of Sports of International Class (according to Russian classification). Their athletic activities averaged 11 ± 1.55 years.

Table 1. *Characteristics of athletes by gender and type of sport*

Type of sport Sport	Male	Female
Ping-pong	1	3
Universal fight	5	4
Tennis	1	1
Golf	-	6
Athletics	1	-
Swimming	1	-
Ski jumping	2	-
Pentathlon	-	3
Synchronized swimming	-	12
Ski cross	1	1
Slopestyle	1	-
Snowboard	1	-
Gymnastics	14	6
Triathlon	-	1
Fencing	1	-
Freestyle	9	1
Hockey	-	2
Field hockey	16	6
Total	**54**	**46**

Risk Factors for Viral Hepatitis

Viral hepatitis is a polyethiological group of diseases. Therefore, the epidemiological role of the source of infection, as well as pathogen transmission mechanisms, are very different and are determined by social, natural, and biological factors.

Results of the analysis of personal data enabled to identify the following main risk factors: surgical operations, tattooing, dental treatment, acupuncture, and injuries (Fig. 1).

The prevailing risk factors for viral hepatitis, both in men and women, were traumatic injuries and dental treatment. In addition, athletes from the analyzed sample visited most countries of Western Europe, the Baltic States, North America, Egypt and China. Therefore, possible infection with HAV and HEV cannot be excluded. All respondents said they had not undergone blood transfusion and had no history of acute viral hepatitis and contacts with infected patients.

Serological Markers of HAV

The problem of HA as a socially significant infection remains urgent for health care in our country. The socio-economic status of the region is essential in understanding the epidemic process of HA. The most vulnerable part of the population includes people living in rural areas and using water from unprotected sources. At the same time, urban residents who use water from centralized water pipelines are no exception since the lack of proper sanitary and epidemiological control over the state of the sewage system can result in an outbreak of HA. Vaccination remains the most effective preventative measure against HA [3].

In 57/66 (86.4%) athletes, IgG antibodies to hepatitis A virus (anti-HAV IgG) were found; predominantly in women — 31/34 (91.2%); one third of whom were engaged in synchronized swimming. Among male athletes, the rate of detection of anti-HAV IgG was 81.3% (26/32). According to questionnaire data, 7 (12.3%) athletes who tested positive for anti-HAV IgG were vaccinated against HA. Anti-HAV IgG were primarily found

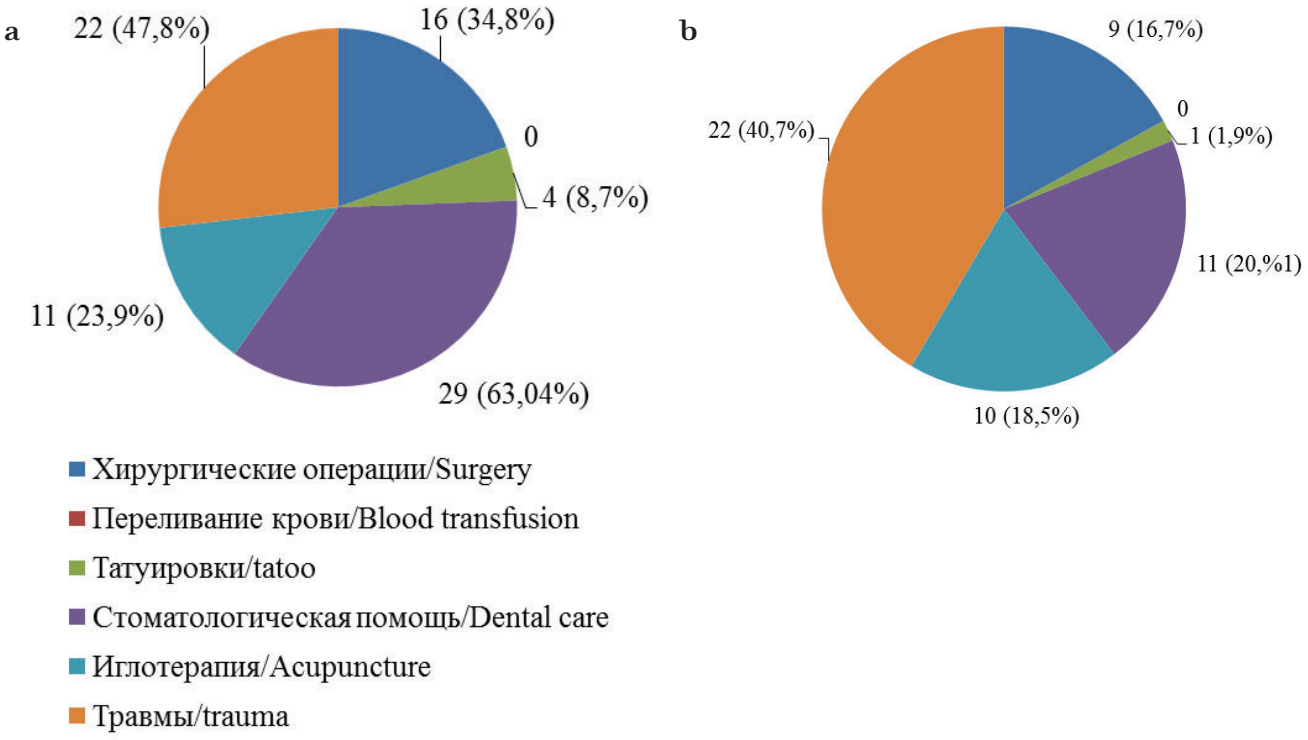

Figure 1. *Risk factors for infection with hepatitis viruses among athletes – women (a) and men (b)*

in the serum of athletes engaged in synchronized swimming and field hockey (average age 16.3 ± 3.8 years).

Relatively high detection rates of anti-HAV IgG 57/66 (86.4%) indicate persistent intensive circulation of HAV and, consequently, a high risk of infection in the population. Information obtained on the immunological structure of HA indicates the need to vaccinate of athletes. This will contribute to the formation of a group immune to HAV, thereby reducing HA prevalence among athletes.

Serological Markers of HEV

The epidemiology of hepatitis E (HE) has undergone several significant changes in recent decades: revealing areas where this infection is endemic (Germany, France, the United States of America, Canada) and registration of local cases there; confirmation of the anthropozoonotic nature of HE; high mortality among pregnant women in the third trimester; chronic forms with prolonged persistence of the virus (more than 6 months) in immunosuppressive individuals [6, 7]. The Russian Federation (RF) has both sporadic (indigenous, local) and outbreak cases. In Russia, the probability of importation of HE is determined by the high level of migration processes, a large influx of tourists from hyperendemic regions with a tropical and subtropical climate [8]. The Russian Federation has kept an official record of HE since 2013.

In our study, the rate of detection IgG antibodies to hepatitis E virus (anti-HEV IgG) in the examined athletes was 3% (2/66) — a man, 19 years old, engaged in freestyle skiing, and a woman, 21 years old, who played golf.

To confirm the latent form of HE, 66 out of 100 athletes were tested for the marker of current infection — IgM antibodies to hepatitis E virus (anti-HEV IgM); all tests were negative.

Due to the lack of vaccination against HE, it is recommended to raise the level of medical and hygienic knowledge and awareness of intestinal infections (including HE) among athletes traveling outside the Russian Federation for training and competitions, especially in countries with a tropical and subtropical climate.

Serological Markers of HBV

No hepatitis B virus surface antigen (HBsAg) was found in any of the 100 blood samples of the examined athletes. The detection rate of IgG antibodies to the HB-core hepatitis B virus antigen (anti-HBcore) in the general group was 13% (13/100) and was slightly higher in women (8/46 (17.4%) and 5/54 (9.1%), respectively). This observation suggests that female athletes have a higher risk of HBV. However, the final conclusion can be made after analyzing the whole group (n = 340).

When analyzing personal data, a group of individuals with a positive history was identified. The following risk factors prevailed in athletes with anti-HBcore: dental treatment — in 5 (38.4%), injuries — in 5 (38.4%), surgical operations — in 4 (30.8%), acupuncture — in 2 (15.4%), tattooing — in 2 (15.4%). There were no differences in risk factors between men and women. The average age of athletes with anti-HBcore was 16.1 ± 2.5 years.

Anti-HBcore was found in blood samples from four athletes engaged in gymnastics, three engaged in freestyle, two — in pentathlon, and one case each among athletes engaged in golf, fencing, field hockey and synchronized swimming.

During DME, none of the examined athletes showed abnormal blood biochemistry.

Only 19 (19%) athletes enrolled in this study were vaccinated (three doses) against HB. None of them had markers of HBV infection (HBsAg, anti-HBcore). The protective level of total antibodies to the HBV surface antigen (anti-HBs) was not assessed at this stage of the study. Therefore, the frequency of vaccination of athletes against HB cannot be assessed too.

The persistence of only anti-HBcore was previously considered as evidence of prior infection with the elimination of virus and disease remission [2]. However, serum HBV DNA levels in anti-HBcore positive patients were usually minimal or found in liver tissue only. Today, antibodies to HBV capsid protein (anti-HBcore) with no HBsAg are considered as a surrogate marker for latent HBV infection. However, no detected HBV DNA in serum cannot exclude latent infection. It should be noted that the diagnostic search for hepatitis etiology limited by the study of HBsAg is ineffective. Therefore, patients with anti-HBcore fall in the group of patients with hepatitis of unknown etiology [9]. Also, such patients are most likely to transmit this infection to others.

The final conclusion on the status of athletes will be made after more complete tests for serological markers of HBV (anti-HBs, anti-HBe), re-study of HBsAg, HBV DNA and fibroelastometry in order to identify and/or assess the stage of liver fibrosis.

Serological Markers of HCV

Hepatitis C (HC) is one of the most important medical and social health problems in many countries, including the Russian Federation. This is a result of significant socio-economic damage, ubiquitousness, severity and active involvement of individuals of reproductive and working age in the epidemic process [10].

According to estimates made by the World Health Organization, there are 71 million people infected with HCV in the world [11]. However, the registered incidence rates for acute and chronic HC do not fully reflect the HCV load on the population. HC may be asymptomatic for decades.

According to our study, only 2% (2/100) of athletes had total antibodies to hepatitis C virus (anti-HCV), which is not higher than the conditional average detection rate (3.5%) of these antibodies in Eastern Europe [11].

Anti-HCV antibodies were found in combination with anti-HBcore in two athletes. In one case, a female athlete, aged 16, was engaged in synchronized swimming, and in another case, — a man, aged 19, was engaged in freestyle. Biochemical parameters of the functional state of the liver (in particular, aminotransferases, bilirubin) did not exceed the upper limit of normal. Ultrasound examination of abdominal organs revealed no pathology.

A test for replication markers, HBV DNA and HCV RNA, was scheduled to exclude latent viral infection.

HBV co-infection with different hepatotropic and non-hepatotropic viruses may be the most significant cause of latent forms of chronic hepatitis [9].

Serological Markers of TTV, TTMDV, TTMV

From 1997 and over the following decade, Japanese virologists (H. Okamoto, T. Nishizawa, M. Ninomiya et al.) discovered viruses whose main characteristic is a gene with a ring structure of a single-stranded DNA molecule [5, 12]. It was only in 2009 that these viruses were registered as a new family of Anelloviridae. Even then, they were known to be extremely prevalent (nearly 100%), not only in humans but also in chimpanzees and African monkeys. This prevalence of Anelloviridae is due to the properties of both parenterally transmitted and enteric viruses. Their infection is thought to be asymptomatic. These viruses are represented by many genera and genotypes (in particular, TTV (genus *Alphatorquevirus*) — 29 genotypes, TTMV (genus *Betatorquevirus*) — 12, TTMDV (genus *Gammatorquevirus*) — 15) [13]. According to taxonomy, several viruses can coexist in a human body. They can cause damage to different organs and systems, but not all of them are related to liver pathology.

Over the past ten years, researchers have not only established the extremely high prevalence of these viruses in many countries but have also confirmed the hepatotropic and hepatopathogenic properties of their several genotypes [14–16].

The nature of chronic liver pathology caused by the viruses of this group is described; electron microscopic images of TTV, TTMDV, TTMV were obtained [16].

However, some researchers are still convinced of the absence of the pathogenicity of the *Anelloviridae* family for humans, believing that their existence in the human body is the result of a long (centuries-old) period of virus and host co-evolution.

According to this study, TTV was found in 86% (86/100) of athletes, TTMDV — in 79% (79/100), and TTMV — in 71% (71/100) (Table 2). The combination of TTV + TTMDV + TTMV was also found in 62% (62/100) of athletes. No abnormal blood biochemistry parameters were revealed.

Analysis of the personal data of athletes in whose serum samples anti-HBcore, anti-HCV and TTV/TTMD/TTMV were detected showed that they were not previously aware of their positive status.

Conclusion

This article presents preliminary results of a study of 100 serum samples obtained during DME from 340 professional athletes engaged in different

Table 2. *Hepatitis Virus Infection Markers among athletes*

Infection markers	Male, n (%)	Female, n (%)
anti-HAV IgG	26/32 (81,35%)	31/34 (91,2%)
Of them:		
HAV vaccinated	3/32 (8,6%)	4/34 (11,8%)
anti-HEV IgG	1/32 (3,1%)	1/34 (2,9%)
anti-HBcore IgG	5/54 (9,2%)	8/46 (17,4%)
Of them:	4/54 (7,4%)	7/8 (87,5%)
anti -HBcore IgG + TTV	1/54 (1,8%)	
anti -HBcore IgG + anti -HCV	+TTV + TTMDV (%)	1/8 (12,5%)
mono TTV	1/54 (1,8%)	3/46 (6,5%)
mono TTMDV	1/54 (1,8%)	1/46 (2,2%)
mono TTMV	2/54 (3,7%)	1/46 (2,2%)
TTV+ TTMDV	2/54 (3,7%)	6/46 (13,0%)
TTMDV + TTMV	4/54 (7,4%)	2/46 (4,3%)
TTV + TTMDV + TTMV	37/54 (6,9%)	25/54 (4,6%)

Note: HA — hepatitis A, anti-HAV IgG — antibodies to hepatitis A virus of class G immunoglobulins, anti-HBcore IgG — antibodies to capsid antigen of hepatitis B virus of class G immunoglobulins, anti-HCV — antibodies to hepatitis C virus, anti-HEV IgG — antibodies to hepatitis E virus of class G immunoglobulins, TTMDV — Torque teno midi virus, TTMV — Torque teno mini virus, TTV — Torque teno virus

sports. A high frequency of detection of hepatitis virus markers was established: HAV in 86.4%, TTV/TTMD/TTMV in 71–86%, HBV in 13%, HEV in 3%, HCV in 2%. Only 7 (12.3%) of 57 athletes were vaccinated against HA.

However, given the positive serological markers and risk factors for infection with hepatitis viruses specified in the questionnaires (dental treatment, surgical operations, injuries, tattoos), there were no reasons for diagnosing previous acute viral hepatitis and information on contacts with patients with hepatotropic infections.

During DME, none of the examined athletes showed abnormal blood biochemistry. However, considering the possible development of latent viral infections in the presence of anti-HBcore and anti-HCV, a molecular genetic test for HBV and HCV replication markers — HBV DNA and hepatitis C ribonucleic acid (HCV RNA) — should be added to the DME protocol.

Due to the high prevalence of viral hepatitis, tests for HBsAg, anti-HBcore, and anti-HCV, as well as information on vaccination, are required when taking children to sports clubs and schools.

Barring athletes from training and competing because of infection with hepatitis viruses is a very difficult decision that often triggers a backlash from the interested federation, relatives and people who have invested enormous moral and material resources in training the athlete.

Vaccine prophylaxis against hepatitis A and hepatitis B is a part of the modern-day strategy for preventing infection and the development of acute viral hepatitis. Its implementation should become a part of the targeted training of athletes to achieve the highest levels in sports.

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Ilchenko L.Yu. (ORCID ID: https://orcid.org/0000-0001-6029-1864): development of research design, analysis of the obtained data, writing and editing text

Morozov I.A. (ORCID ID: https://orcid.org/0000-0002-3864-2848): research design, text editing

Kozhanova T.V.: determination of hepatitis virus infection markers; literary search

Soboleva N.V.: determination of markers of hepatitis virus infection; statistical data processing text writing

Melnikova L.I.: collection of material, analysis of the data

Kruglova I.V.: collecting material; analysis of the results

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