HEPATITIS B, C AND TTV VIRUS INFECTION IN HIGHLY TRAINED ATHLETES

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Biomedical support is aimed at provision of the athletes' training at various stages of the training and competition process. Withholding of access to this process due to health problems resulting from hepatitis virus infection is a demanding task. The study was aimed to assess the detection rate of the hepatitis B virus, hepatitis C virus and TT virus infection markers in highly trained athletes. A total of 384 blood serum samples were collected from 240 males and 144 females aged 14–49 (athletes engaged in playing sports, precision sports, technical sports, etc.) within the framework of the multicenter open-label cross-sectional clinical trial. All athletes answered a questionnaire, which included demographic information, characteristics of sports, information about the infection risk factors, information about the fact of past acute viral hepatitis and vaccination. Markers of infection with hepatitis B virus, hepatitis C virus and TTV were identified in blood serum by enzyme immunoassay. HbsAg was detected in two surveyed athletes. Anti-HBcore (surrogate marker of latent HBV infection) was detected in 7% of samples (27/384); 1% of athletes (4/384) had a positive hepatitis C virus total antibody test (anti-HCV). Anti-HCV in combination with anti-HBcore was detected in one female athlete (14 years of age, tennis player). DNA of TTV, TTMDV and TTMV was detected in blood serum samples of 89.1%, 83.1% and 85.4% of athletes, respectively. High detection rate of the hepatitis virus markers was observed.

Keywords: highly trained athletes, hepatitis B, hepatitis C, hepatitis TTV, virus markers

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Compliance with ethical standards: the study was approved by the Ethics Commitee of the Clinical Hospital No. 85 of FMBA of Russia (protocol № 157 dated September 19, 2018). All subjects submitted the informed consent to blood sampling, identification of the hepatitis virus infection markers, and publication of results.

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ИНФИЦИРОВАННОСТЬ ВИРУСАМИ ГЕПАТИТОВ B, C И TTV ВЫСОКОКВАЛИФИЦИРОВАННЫХ СПОРТСМЕНОВ

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Медико-биологическое сопровождение направлено на обеспечение подготовки спортсменов в различные периоды тренировочно-соревновательного процесса, отказ от допуска к нему из-за отклонений в состоянии здоровья вследствие инфицирования вирусами гепатитов является достаточно трудной задачей. Целью исследования было оценить частоту выявления маркеров инфицирования вирусами гепатитов B, C и TTV у высококвалифицированных спортсменов. В многоцентровом открытом одномоментном клиническом исследовании у 240 мужчин и 144 женщин в возрасте 14–49 лет (спортсменов игровых, сложнокоординационных, технических и других видов спорта). Получено 384 образца сыворотки крови. Все спортсмены заполняли анкету, включавшую демографические данные, характеристику вида спорта, сведения о факторах риска инфицирования, информацию о наличии перенесенного острого вирусного гепатита и вакцинопрофилактике. В сыворотке крови методом иммуноферментного анализа определяли маркеры инфицирования вирусами гепатитов B, C и TTV. У двух из обследуемых спортсменов выявлен HBsAg. В 7% (27/384) образцов обнаружены anti-HBcore (суррогатный маркер латентной HBV-инфекции), у 1% (4/384) спортсменов — суммарные антитела к вирусу гепатита C (anti-HCV). Anti-HCV был выявлен в сочетании с анти-HBcore у одной спортсменки (возраст — 14 лет, занимается теннисом). В образцах сывороток крови 89,1%, 83,1%, 85,4% спортсменов обнаружены DNA TTV, TTMDV и TTMV соответственно. Установлена высокая частота обнаружения маркеров вирусов гепатитов.

Ключевые слова: высококвалифицированные спортсмены, маркеры вирусов, гепатит B, гепатит C, гепатит TTV

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Sport is one of the components of physical fitness, while high performance (elite) sport is an activity aimed at reaching peak athletic performance, which requires mobilization of emotional resources and body's functional capacity.

Peak athletic performance is reached due to the scientific and methodical support of the athlete training system at various stages of the training and competition process. Biomedical support involves investigation of the athletes' competitive activity together with the serial comprehensive, routine and detailed medical examinations (DME) performed in accordance with the specially designed programs in medical institutions [1, 2].

Viral hepatitis is a diffuse inflammatory liver disease caused by hepatotropic viruses. The infection has a variety of transmission routes and epidemiologic features [3].

Over the past 30 years, at least five different hepatitis viruses have been discovered: A (HAV), B (HBV), delta or D (HDV), C (HCV) and E (HEV) [3]. There is emerging evidence of the existence of other hepatotropic viruses capable of playing a role in the pathogenesis of both acute and chronic liver disease (CLD). Thus, hepatitis G virus (*Pegivirus*), TT virus and SEN virus were isolated from the samples collected from patients with acute or chronic hepatitis (CH) [4]. However, virological and epidemiological studies made it impossible to define the role of these viruses in the pathogenesis of the disease [3, 4].

Hepatitis B and C viruses remain the most important etiological factors of liver disease. Thus, in 2020 the acute hepatitis B infection incidence per 100,000 population was 0,35; the value for chronic hepatitis B infection was 4.4; the incidence of acute hepatitis C infection was 0.66; the value for chronic hepatitis C infection was 16.7 [5]. There are no official recors of infection caused by hepatitis delta virus and *Torque teno virus* (TTV) in our country.

Taking into account the global prevalence of viral hepatitis it can be affirmed that athletes are also at risk of infection. They could be infected during the training session or the rest period. Athletes may get infected with viral hepatitis owing to activities not related to sports. However, there is some risk of virus transmission when practicing some sport. Furthermore, the team sport athletes maintain close contact with the team members for a long time; sharing food and drinks could increase the likelihood of the hepatitis virus fecal-oral transmission [6]. The actual prevalence of viral hepatitis among athletes is unknown [6, 7].

In this regard, questions arise about safety of sport and participation of athletes in the competitions after the diagnosis of acute or chronic viral hepatitis. Sports physicians should decide on the terms of the athlete's admission to training and competitions, understand the risk of viral transmission during sport, and be able to advise the athletes accordingly. Finally, specialists, who take care of the athletes' health, should be familiar with the strategies of the viral hepatitis prevention [8].

Athletes much more often get infected with HBV and HCV during activities not related to sport. These are unprotected sexual intercourse; the use of injection drugs, such as psychoactive substances and anabolic steroids; sharing personal items (for example, razors, toothbrushes, nail nippers). The cases of infection during tattooing, piercing and body painting have been reported [9].

Furthermore, healthcare professionals should consider the risk of the HBV and HCV horizontal transmission in athletes [10]. When playing sports, the infected blood could contaminate skin or mucous membranes of the other team members or individuals who come into contact with them [10].

The study was aimed to assess the detection rate for the HBV, HCV and TT infection markers in highly trained athletes.

METHODS

The multicenter open-label cross-sectional clinical trial was performed in the Center for Diagnosis and Management of Viral Hepatitis of the Clinical hospital No. 85 of FMBA of Russia and Federal Scientific and Clinical Center for Sports Medicine and Rehabilitation of FMBA of Russia.

Blood samples were collected from 384 athletes during the detailed medical examination. Inclusion criteria: highly trained athletes; age 14–49 years; informed consent to blood sampling, identification of the viral hepatitis markers, and publication of results.

All athletes completed the questionnaire, which had been developed by the researhers (Table 1).

Markers of viral hepatitis were identified in blood samples. Serological markers of HBV and HCV infection (hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBcore), hepatitis C virus antibody (anti-HCV)) were identified by enzyme immunoassay using the number of test systems in accordance with the manufacturers' instructions. The following test systems were used: DS-EIA-HBsAg-0.01; DS-EIA-HBsAg-0.01 for confirmation; DS-EIA-anti-HBc; DS-EIA- SPECTRUM-G, DS-EIA-ANTI-HCV-SPECTRUM-GM (Diagnostic Systems; Russia).

Deoxyribonucleic acid (DNA) of viruses of the family *Anelloviridae* was detected in blood serum samples by polymerase chain reaction (PCR). Isolation of nucleic acids from the blood serum samples was performed using the kit for isolation of deoxyribonucleic acid/ribonucleic acid (DNA/RNA) from blood serum or plasma on the MP@SiO_2 magnetic particles (Sileks; Russia) in accordance with the manufacturer's protocol.

DNA of viruses of the family Anelloviridae in blood serum was identified by nested PCR [4], allowing one to distinguish between TTV, Torque teno midi virus (TTMDV), and Torque teno mini virus (TTMV) based on the amplified fragment size. The amplification product size was 112–117 nucleotides for TTV, 88 nucleotides for TTMDV and 70–72 nucleotides for TTMV. The product with the length of 207 base pairs (bp) was defined by 2% agarose gel electrophoresis conducted with TBE (Tris-borate-EDTA) buffer.

Statistical analysis of raw data involved calculation of the main selected indicators for quantitative variables. The detection rate of the serological markers (HBsAg, anti-HBcore, anti-HCV) and Anelloviridae virus DNA was calculated as a percentage of their total quantity in the sample.

RESULTS

The sample of athletes, who's blood samples were included in the study (384 out of 384), was randomized and represented by athletes engaged in different sports (playing sports, precision sports, technical sports, etc.) (Table 2).

Markers of HBV infection

Assessment of 384 blood samples revealed HBsAg in two individuals (male, fencing; female, volleyball). The detection rate of anti-HBcore in the general group was 7% (27/384). Female athletes were at higher risk of HBV infection compared to male athletes (18/144 and 9/240, respectively).

Analysis of personal data revealed the group of individuals with positive history. The following risk factors for infection predominated among athletes with anti-HBcore: dental care in 16 individuals (59.3%), injuries in 8 (29.6%), surgery in

Table 1. Questionnaire for highly trained athletes

Full name	Fill in or underline
Age (completed years)	
Gender	male / female
Place of birth	
Athletic discipline	
Athlete's qualification	
History of acute viral hepatitis	A, B, C, E (when?)
Vaccination against HAV	Yes, no (when?)
Vaccination against HBV	Yes, no (when?)
Surgical interventions	Yes, no (when?)
Transfusion of blood or blood substitutes	Yes, no (when?)
Dental care	Yes, no (when?)
Tattoos, piercings	Yes, no (when?)
Acupuncture	Yes, no (when?)
Traveling to foreign countries (which exactly?)	
Exposure to individuals with viral hepatitis	
Date of blood sampling	

Note: HAV — hepatitis A virus, HBV — hepatitis B virus.

9 (33.3%), acupuncture in 2 (7.4%), and tattooing in one individual (3.7%). There were no defferences between males and females based on the risk factors. The average age of athletes with anti-HBcore was 16.1 ± 2.5 years.

Anti-HBcore was detected in blood serum of four artistic gymnasts, eight field hockey players, two pentathlon athletes, two hockey players, two golf players, two swimmers; single positive samples were obtained from freestyle skier, golf player, fencer, tennis player, artistic gymnast, universal martial arts fighter, and gun shooting athlete.

According to their personal data, only 11.7% of athletes (45/384) were vaccinated (tree doses) against HBV. However, HBV infection markers (anti-HBcore) were found in one of them (female, artistic gymnastics). At this stage of the study, evaluation of the protective levels of the total hepatitis B surface antibody (anti-HBs) in athletes has not been completed.

The following was observed in HbsAg-positive patients: HBV DNA, viremia level exceeding 2000 IU/mL, elevation of alanine aminotransferase levels up to 1.5 of the reference limit (upper limit of normal), stage 1 fibrosis based on the transient elastography data. After 12 weeks of therapy with entecavir given at a dose of 0.5 mg/day, undetectable levels of HBV DNA were achieved. Therapy and follow-up were resumed.

Markers of HCV infection

According to the study, anti-HCV were found only in 1% of athletes (4/384). Combination therapy with sofosbuvir 400 mg/day and daclatasvir 60 mg/day was started after additional testing, which included assessing HCV RNA, viremia level, activity of chronic hepatitis C, and fibrosis stage. At the time of writing the article, the athletes had been followed up for 1.5–2 years after the antiviral therapy completion and achieving sustained virologic response.

Anti-HCV in combination with anti-HBcore were found in one female tennis player (aged 14). Biochemical indicators characterizing liver function (particularly, aminotransferases, bilirubin) did not exceed the upper limit of normal range. Abdominal ultrasound showed no abnormalities. Markers of viral replication, HBV DNA and HCV DNA, were assayed in order to exclude latent viral infection, the negative test result was obtained. The athlete was further followed-up.

Markers of TTV, TTMDV, TTMV infection

According to the study, TTV was detected in 89.1% of athletes (342/384), TTMDV in 83.1% (319/384), and TTMV in 85.4% of athletes (328/384) (Table 3). The combination TTV + TTMDV + TTMV was detected in 69% of athletes (265/384). Biochemical indicators were within normal range.

DISCUSSION

The article presents the results of a study of 384 blood serum samples obtained from 384 highly trained atheletes engaged in different sports. The detection rate of the hepatitis virus markers was high: 89.1% for TTV, 83.1% for TTMDV, 85.4% for TTMV, 7% for HBV, 1% for HCV.

It must be pointed out that the diagnosis of the hepatitis etiology limited to testing for HbsAg is ineffective, which allows patients with anti-HBcore to join the group of individuals with hepatitis of unknown etiology [4, 11]. Furthermore, such patients are most likely to infect other people.

Previously, only the presence of the stably detectable anti-HBcore was considered the sign of the past infection followed by the virus elimination and the disease remission [2]. However, anti-HBcore-positive patients generally have minimum serum levels of HBV DNA or HBV DNA that could be detected only in liver tissue. Today, the presence of anti-HBcore and the absence of HBsAg are considered the surrogate marker of latent HBV infection, and the lack of detectable HBV DNA in blood serum is not considered the fact precluding the infection.

Thus, the final judgement on the status of athletes with HbsAg is established through identification of the broader range of the HBV serological markers (anti-HBs, anti-HBe), confirmatory test for HbsAg and HBV DNA, and transient elastography aimed at detecting liver fibrosis and/or assessing the stage of fibrosis.

Along with HBV infection, hepatitis C virus (HCV) remains highly relevant. It is the major health and social issue in many of the world's countries, including the Russian Federation, owing

Table 2. Characteristics of athletes by gender and athletic discipline

Athletic discipline	Males	Females
Tennis	15	5
Universal martial arts	8	4
Biathlon	4	4
Bobsleigh	9	–
Volleyball	–	3
Canoe slalom	15	1
Golf	1	6
Judo	1	2
Luge	1	–
Track and field	12	10
Swimming	4	4
Jumping	3	1
Pentathlon	–	3
Artistic swimming	–	12
Ski cross	1	1
Slopestyle	1	–
Snowboard	1	–
Artistic gymnastics	15	6
Gun shooting sports	17	13
Sumo	5	10
Short-track speed skating	10	6
Football	–	12
Triathlon	–	1
Fencing	24	11
Freestyle skiing	19	8
Hockey	27	2
Field hockey	47	18
Chess	–	1
Total	240	144

to significant social and economic harm, global incidence, disease severity, and active involvement of people of working and childbearing age in the epidemic process [12].

According to the World Health Organization, the estimated number of people infected with HCV all over the world is 71 million [3]. However, the reported incidence rates for acute and chronic HCV do not fully reflect the population infection rate. HCV could remain asymptomatic for decades.

In our study, the detection rate of the most commonly identified HCV marker, anti-HCV, in athletes (1%) does not exceed the conditional average detection rate (3.5%) obtained for this antibody in Eastern Europe [3] and, therefore, suggests low prevalence of HCV in the country. However, HCV co-infection with different hepatotropic and non-hepatotropic viruses could become the major underlying cause of the chronic hepatitis latency [4].

A decade after 1997, Japanese virologists H. Okamoto, T. Nishizawa, M. Ninomiya, et al, discovered viruses with genomes composed of one circilar single-stranded DNA molecule [13, 14]. In 2009, the viruses were classified as a new family *Anelloviridae*. Already then we knew about the extremely high prevalence of viruses, which reached 100%

Table 3. Markers of hepatitis virus infection in athletes

Infection markers	Males, *n* (%)	Females, *n* (%)
anti-HBcore IgG Of those: anti -HBcore IgG + TTV anti -HBcore IgG + anti-HCV	9/240 (3,8%) 9/9 (100%) 0/9 (0%)	18/144 (12,5%) 14/18 (77%) 1/18 (5,5%)
mono anti-HCV	3/240 (0,8%)	1/144 (1,44%)
mono TTV	214/240 (89,2%)	128/144 (88,9%)
mono TTMDV	199/240 (82,9%)	120/144 (83,3%)
mono TTMV	206/240 (85,8%)	122/144 (84,7%)
TTV + TTMDV	184/240 (76,7%)	109/144 (75,7%)
TTMDV + TTMV	178/240 (74,2%)	106/144 (73,6%)
TTV + TTMDV + TTMV	168/240 (70%)	97/144 (67,4%)

not only in humans, but also in chimpanzee and African green monkeys. Such prevalence of viruses of the family *Anelloviridae* results from the properties of the parenterally transmitted and enteric viruses. It is believed that infection with these viruses is asymptomatic. The viruses are represented by numerous genera and genotypes (particularly, there are 29 genotypes of TTV (*genus Alphatorquevirus*), 12 genotypes of TTMV (*genus Betatorquevirus*), 15 genotypes of TTMDV (genus Gammatorquevirus)) [15]. Several viruses can coexist in the human body. Viruses are capable of affecting various organs and systems, but not all of them are related to liver disorders.

Over the past decade, the researchers observed not only the extremely high prevalence of these viruses in the populations of many countries, but also confirmed hepatotropism of some genotypes and their pathogenicity in the liver [15, 16].

The nature of chronic liver disease caused by viruses of this group has been described, the electron micrographs of TTV, TTMDV, TTMV have been obtained [17]. However, some researchers still believe in the non-pathogenic persistence of Anelloviridae family in humans assuming that their presence in human body results from centuries of the virus–host coevolution.

Unfortunately, English medical literature provides no data on the athletes' infection, which makes it impossible to perform analysis and compare the results with the data of similar foreign studies.

Analysis of personal data of the surveyed elite athletes, in who's serum samples anti-HBcore, anti-HCV and TTV/TTMD/TTMV had been detected, showed that the athletes were unaware of their positive status.

All the above points to the need for asessment of the wider range of viral infection markers in athletes without being limited to testing for HBsAg and anti-HCV. Co-infection with different hepatotropic and non-hepatotropic viruses, mutual influence of viruses, and their role in the development and progression of chronic liver diseases are the essential aspects of research.

CONCLUSIONS

Athletes are succeptible to the same viral infections as other representatives of human population, including infection with hepatitis viruses [18]. The risk of HBV and HCV infection spread does exist. However, the prevalence of these infections is not as high. Acute viral hepatitis in athlete does not require the long-term limitation of physical activity. Athletes diagnosed with acute hepatitis are under appropriate medical supervision aimed to prevent liver disease progression and chronification. In case of chronic hepatitis, efficient advanced therapeutic approaches make it possible to achieve the complete HCV elimination or clinical recovery in patients with chronic HBV infection. The prevalence of viral hepatitis in the population is high, that is why it is necessary to perform testing for the HBsAg, anti-HBcore and anti-HCV markers and acquire the data on vaccination against HAV and HBV when selecting children who would go to the sports sections and schools. Withholding of access to training and competition processes due to health problems resulting from hepatitis virus infection is a demanding task that elicits a negative reaction of the participating federation, relatives and people, who had used great moral and material resources to train the elite athlete. Currently, preventive vaccination against viral hepatitis is considered a modern strategy for prevention of infection and acute viral hepatitis progression. Vaccination should become a part of targeted training of athletes in order to reach peak athletic performance.

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