ВИРУСОЛОГИЯ

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QUANTITATIVE HBSAG LEVEL IN THE TRANSFORMATION OF CHRONIC HEPATITIS B WITH DELTA AGENT INTO LIVER CIRRHOSIS

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XULOSA

Gepatit B virusi butun dunyoda global ahamiyatga ega, chunki u sirroz va gepatotsellyulyar karsinomaga o'tish xavfi bilan surunkali jigar kasalligini keltirib chiqaradi. HBsAg miqdorini aniqlovchi to'plamlarining paydo bo'lishi surunkali gepatit B-da davolanishga javobni aniqlash va natijalarni bashorat qilish uchun muhim ahamiyat kasb etadi.

Tadqiqot maqsadi. *Surunkali delta agentli HBV infektsiyasini monitoring va bashorat qilishda HBsAg ro'lini baholash uchun miqdoriy HBsAg ko'rsatkichlari va HDV RNK darajalari o'rtasidagi bog'liqlikni tahlil qilish.*

Materiallar va usullar. *Mikst HBV va HDV infektsiyasi bo'lgan 30 bemordan zardob namunalari eg'ildi. Ulardan delta agentli surunkali virusli gepatit B 15 nafar bemor va HBV+HDV etiologiyali jigar sirrozi 15 nafar bemorda kuzatilgan.*

Natijalar. *Jigar sirrozi kuzatilgan bemorlar qonida o'rta va yuqori darajadagi virus yuklamasi aniqlangan; faqat bitta bemorda 1,5x103 XB/ml past virus yuklamasi kuzatildi. O'rtacha va yuqori virus yuklamalarida HDV RNK ko'rsatkichlarida sezilarli farqlar kuzatildi, HBsAg ko'rsatkichlari esa sezilarli farqlarni ko'rsatmadi.*

Xulosa. *HDV infektsiyasida HBsAg miqdoriy ko'rsatkichlarining qondagi gepatit D virusi miqdori bilan bog'liqligi kuzatilmadi. Shunday qilib, zardobdagi HBsAg miqdori va HDV RNK darajasi o'rtasida hech qanday bog'liqlik aniqlanmadi.*

Kalit so'zlar: *HDV infektsiyasi, HBsAg miqdori, jigar sirrozi, HDV RNK.*

РЕЗЮМЕ

Вирусный гепатит В имеет глобальное значение во всем мире, поскольку он вызывает хроническое заболевание печени с риском перехода в цирроз печени и гепатоцеллюлярной карциномы. Появление коммерческих доступных наборов для количественного определения HBsAg представляет растущий интерес для определения ответа на лечение и прогноза исхода при хроническом гепатите В.

Цель исследования. *Анализировать взаимосвязь количественных показателей HBsAg с уровнем РНК HDV для оценки роли HBsAg в мониторинге и прогнозировании хронической инфекции HBV с дельта-агентом.*

Материалы и методы. *Образцы сыворотки были взяты у 30 пациентов со микст инфекциями HBV и HDV. Из них было 15 пациентов с хроническим вирусным гепатитом В с дельта агентом и 15 пациентов с циррозом печени HBV+HDV этиологии.*

Результаты. *У пациентов с циррозом печени в крови определяли средний и высокий уровень вирусной нагрузки; только у одного пациента была низкая вирусная нагрузка 1,5x103 МЕ/мл. Наблюдались значительные различия РНК HDV при средней и высокой вирусной нагрузке, в то время как показатели HBsAg не демонстрировали значимых различий.*

Выводы. *При HDV инфекции количественные показатели HBsAg не имели достоверной связи с количеством вируса гепатита D в крови. Таким образом, не было обнаружено никакой связи между концентрацией HBsAg в сыворотке и уровнем РНК HDV.*

Ключевые слова: *HDV инфекция, уровень HBsAg, цирроз печени, РНК HDV.*

Chronic hepatitis D (CHD) is a severe and rapidly progressive form of chronic viral hepatitis, leading to liver cirrhosis (LC) in 70% of cases within 5-10 years. The initial stages of formation of the liver cirrhosis can be asymptomatic or with minimal signs, which complicates its diagnosis [1,2,3]. In 15% of patients, LC may develop within 1 to 2 years from the onset of acute hepatitis. The risk of developing LC is three times higher in patients infected with HDV infection compared to patients with HBV monoinfection [4,5,6,7,8]. In a study conducted in

Greece, it was shown that in patients with CHD who did not die from liver failure, the risk of developing hepatocellular carcinoma (HCC) within 12 years was almost 42% [9, 10]. Latent HDV infection is characterized by the detection of markers of active HDV replication only in liver tissue (HDV RNA, HDAg), while anti-HDV can be detected in blood serum in the absence of HBV DNA. The presented form of delta infection was first described in patients who underwent orthotopic liver transplantation for viral LC [5,10].

The quantitative determination of HBsAg has become an innovation in the determination of hepatitis B markers [11]. HBsAg can serve as a serological marker for HBV infection [12]. Studies conducted in various countries have shown a wide range of HBsAg values in patients with chronic HBV infection and a fairly strong correlation between HBsAg levels, HBeAg detection and blood DNA HBV levels [13,14,15]. A part of the studies devoted to the determination of the HBsAg content in the blood have demonstrated a positive correlation between the HBV viremia level and the HBsAg level concentration [16,17]. Brunetto et al., [18] tried to find such a threshold value of HBsAg concentration, below which in patients with D-genotype inactive stage does not progress to active stage. There are suggestions that low HBsAg concentrations (<500 U/mL) allow a 100% certainty of diagnosis of inactive carriage without additional monitoring. However, such studies are few and contain a small number of observations. Currently, the role of HBsAg quantification for the diagnosis of HBV infection requires clarification [19]. Ozaras R. et al. stated that quantification of hepatitis B surface antigen (HBsAg) is a surrogate marker associated with HBV DNA level [20]. In a study focused on the value of HBsAg quantification in serum detected that HBsAg concentration corresponds to viral load [21]. A number of authors have used HBsAg quantification to determine the efficacy of etiotropic therapy against hepatitis B virus [16, 22, 23]. It is assumed that in case of low viral load but high HBsAg concentration the prognosis of treatment is unfavorable [24].

THE AIM OF THE STUDY

To analyze the relationship of HBsAg quantification with HDV RNA levels to evaluate the role of HBsAg in monitoring and predicting chronic HBV infection with delta agent.

MATERIALS AND METHODS

Clinical samples and participants

30 patients with mixed HBV- and HDV-infection who applied to the clinic of the Research Institute of Virology were examined and blood serum samples were collected. Prior to the study, blood samples were stored at -70°C. There were 15 patients with chronic viral hepatitis B with delta agent aged 26 to 45 years (mean age - 35,0±1,5 years), with liver cirrhosis of HBV+HDV etiology 15 patients aged 28 to 60 years (mean age - 38,2±1,8 years). Among them, there were 13 men (43.3%) and 17 women (56.7%). All patients satisfied to the diagnostic criteria for chronic hepatitis and LC and did not receive antiviral therapy. Inclusion criteria for the study were the presence of HBsAg and HDV RNA in serum.

Enzyme-linked immunosorbent assay

The etiologic diagnosis was established on the basis of enzyme-linked immunosorbent assay (ELISA) results. For detection of surface antigen of hepatitis B virus, antibodies to hepatitis C virus and antibodies to hepatitis Delta virus in serum, we used the kits "DS-ELISA-HBsAg", "ELISA-anti-HCV" and "ELISA-anti-HDV", respectively, produced by Diagnostic Systems (Russia, Nizhny Novgorod). Quantitative determination of HBsAg in serum was performed by ELISA using the VECTOR-BEST kit (Novosibirsk, Russia).

Real-time polymerase chain reaction

Molecular genetic studies included determination of HBV DNA and HDV RNA in blood by PCR using AmpliSens HBV-FL and AmpliSens HDV-FL kits (Russia) in a Rotor-Gene Q thermocycler. The amplification conditions for detecting of HBV DNA were as follows: 95°C-15 min; 45 PCR cycles (95°C-2 sec, 60°C-10 sec); 10°C storage. For the detection of HDV RNA the amplification conditions were as follows: 50°C-30 min; 95°C-15 min; 45 PCR cycles (95°C-2 sec, 60°C-10 sec); 10°C storage.

Statistical analysis

Statistical processing of the research results was carried out using the Student's test. Differences were considered significant at $p<0.05$.

RESULTS

A comparative analysis of HBsAg quantification in CHB with delta agent and LC of HBV and HDV etiology was performed. To determine the relationship between HBsAg and HDV RNA levels in blood, we categorized viral load into high, medium and low. The relationship between quantitative HBsAg level and HDV RNA level among the observed patients was analyzed. The results are summarized in Table 1. The mean values of HDV RNA and HBsAg were determined at different HDV RNA levels in patients with CHB+CHD.

Table 1

Blood HBsAg levels at different HDV RNA levels in patients with CHB with delta agent

Indicators	Viral load			P	P1	P2
	low	medium	high			
HDV RNA	297.5±13.2	394500±178.9	7360000±2095614	<0.001	<0.005	<0.001
HBsAg	0.114±0.05	2.79±0.38	3.68±0.75	<0.005	>0.05	<0.005

Note: - significance of differences between low and medium viral loads.

- significance of differences between medium and high viral loads.

- significance of differences between low and high viral loads.

The presented data clearly show that HBsAg levels changed at different viral load levels. HBsAg concentration was related to HDV RNA level. Our study showed that HDV RNA load was positively associated with HBsAg level, and serum HBsAg could reflect the degree of viral activity as well as viral replication and the degree of disease progression. HBsAg quantification can be recommended for routine use to monitor viral load in CHB with delta agent. Depending on HBsAg quantification, an increase or decrease in viral load can be predicted. Table 2 summarizes the mean HBsAg levels in patients with liver cirrhosis of HDV etiology.

Table 2

HBsAg quantification in patients with LC with different levels of HDV RNA

Indicators	Viral load		P
	medium	high	
HDV RNA	189818±77478	20966668±2521	<0.001
HBsAg	3.0±0.52	3.56±1.46	>0.05

Note: P - significance of differences between medium and high viral load.

Patients with liver cirrhosis had medium and high viral load levels; only one patient had a low viral load of $1.5x10^3$ IU/mL. There were significant differences between HDV RNA levels at medium and high viral load, while HBsAg levels were not significantly different. This indicates that in LC HBV and HDV etiology, HBsAg quantification did not have a significant relationship with hepatitis D virus levels. Thus, no relationship was found between serum HBsAg concentration and HDV RNA levels.

DISCUSSION

Currently, the quantification of serum HBsAg in CHB patients has attracted widespread attention [25-27]. It has been suggested that serum HBsAg levels may reflect viral activity, which is consistent with the results of relevant studies [25]. Several studies have been published in the last few years on the diagnostic value of quantitative HBsAg determination for clarifying the clinical variant of chronic HBV infection and its monitoring. There is speculation that a low HBsAg concentration (<500 U/mL) allows inactive carriers to be identified with 100% certainty without additional monitoring. It is assumed that in low viral load but high HBsAg concentration the prognosis of treatment is unfavorable [28].

The results of our study showed that patients with liver cirrhosis had medium and high viral load levels in the blood; only one patient had a low viral load of $1.5x10^3$ IU/mL. There were significant differences between HDV RNA levels at medium and high viral load, while HBsAg levels were not significantly different. This indicates that in LC HBV and HDV etiology, HBsAg quantification did not have a significant relationship with hepatitis D virus levels.

Thus, high HBsAg levels are considered predictors for prognosis of process activity in CHB with delta agent and can be a prognostic indicator of possible transformation of CHB with delta agent into LC. Evaluating the above, we can conclude that determination of quantitative HBsAg in blood has an important diagnostic and prognostic significance. In addition, the results of our study showed that in case of negative HBV DNA in blood (PCR) it is possible to determine the state of HBV infection by the level of serum HBsAg, thus influencing the choice of optimal therapy.

CONCLUSIONS

1. In CHB patients with delta agent, the level of HBsAg in the blood changes with different viral load parameters.

2. Depending on the quantitative HBsAg it is possible to predict of dynamics of change of viral load in patients with HDV infection.

3. To assess the intensity of surveillance of CHB+D patients, the use of HBsAg can be recommended.

4. In liver cirrhosis, the isolated determination of quantitative HBsAg does not allow to control and predict the level of HDV RNA in the blood, therefore, quantitative HBsAg indices cannot have diagnostic and prognostic significance in patients with LC.

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