Biomarkers Used In The Diagnosis Of Chronic Hepatitis B Virus Infection And New Biomarkers

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***Abstract*—Hepatitis B virus (HBV) leads to HBV infection by causing chronic liver damage in humans. New biomarkers are needed to diagnosis and follow up of infection and follow up of treatment response. It is aimed to give information about new biomarkers through this study.**

Keywords—Hepatit B virus; Diagnosis; New Biomarkers.

I. INTRODUCTION

HBV is one of the major global health problems, which causes more than one million deaths worldwide each year by causing chronic damage to the human liver [1].

Today, only 10% of the Hepatitis B patients in the world can be diagnosed [2]. To diagnose those infected by HBV, HBV surface antigen (HBsAg) is detected, the serum level of surface antibody (anti-HBs) formed against surface antigen and of the antibody (anti-HBc) formed against core protein are identified; moreover, to detect the phase of the infection and to start the treatment, HBV e antigen (HbeAg) expressing the virus replication and the level of the antibody (anti-Hbe) formed agaist that are detected [3]. In addition to that, HBV DNA which is a nucleic acid belonging to the virus, and with this, following the level of the alanine aminotransferase are especially used in follow-up and management of Hepatitis B patients [4, 5], in the decision of treatment of anti-HBV and in the follow-up of the treatment response [3].

History of Hepatitis B Virus and Genome Structure

Blumberg and workmates of him identified HBsAg antigen belonging to HBV in an Australian Aboriginal patient in 1967 [6], and in 1970 Dane and workmates identified the complete virus particle and visualized it with electron microscope [7].

HBV is an enveloped virus [8] with hepatotrope, icosahedral symmetry, circular and partially double-stranded DNA [9], genome length 3.2 kilobytes [10], belonged to the genus Orthohepadnavirus, Hepadnaviridae family. There is HBsAg outermost of the virus, HBV core antigen (HBcAg) under it, HBeAg (found in all members of the Hepadnaviridae family) innermost part of it [11]. There are 4 gene regions belonging to the virus as X, S, P, C. HBsAg by S gene, HBcAg by C gene, HBV DNA by P gene, capsid proteins are encoded by X gene [11]. Missing virus particles are important features of the virus [12]. Only HBsAg consists of these empty capsids. Missing virus particles do not cause infection but cause antibody production [12].

Diagnostic Procedures of Hepatitis B Virus

In the diagnosis of HBV infection and in monitoring of the patients, antigens, antibodies and existence of HBV DNA (if any, its level) which is a viral nucleic acid are detected; moreover, ALT level of the patient is followed-up [3].

Rapid diagnostic tests: It is based on the principle of detecting HBV found in blood or saliva with small devices and/or detecting of the antibodies formed in the body against the virus [13]. Although some of these tests are approved by World Health Organization [14], American Association for the Study of the Liver Diseases (AASLD) and The European Association for the Study of the Liver (EASL) have not approved [3]. Due to the fact that these tests are cheap and easily accessible, they are more appropriate for people who cannot access medical care, such as drug users, the homeless [3].

Detecting of HBsAg; HBsAg, which is the specific and delicate biomarker of HBV infection, is an envelope protein of the virus, takes a role in the escape of the host from immune system, is of episomal mini chromosome (cccDNA) origin [15, 3]. HBsAg may differ depending on the phase of infection and its genotype; it is detected in serum 1-2 weeks before clinical symptoms appear [3]. It can be detected in the blood approximately 4 weeks after contact with the virus, its presence states active infection. Symptoms appear, serum transaminases increase and anti-HBc IgM can be detected approximately 12 weeks (between 9-21 weeks) after the contact. HBsAg disappears within 2-6 months in acute Hepatitis B cases and a window period occurs until antibodies are detected. Only serological marker that can be detected in this period is anti-HBc-IgM. Window period starts with the beginning of the symptoms and can continue until 6-9 months after the infection. After the window period, anti- HBs antibodies are produced and detected. Continuation of HBsAg positivity for 6 months or longer indicates that the infection has become chronic [16].

Detection of Anti-HBs; Anti-HBs antibody is a neutralizing antibody indicating whether there is immunity against HBV [3]. Anti-HBs antibody is both the only serological marker of the immunity acquired

by vaccination and expresses the natural infection, likewise, HBV infected persons have it with anti-HBc IgG simultaneously [16].

Detection of HBeAg; HBV core protein encodes HBeAg excreted from the infected hepatocyte and expressing active virus replication and high infectiousness [3]. HBeAg appears soon after from HBsAg, can be detected 6-12 weeks after exposure to HBV and expresses high HBV DNA level and high infectivity [3]. In window period, HBeAg and anti-HBe are negatives. Seroconversion from HBeAg to anti-HBe is an indicator of the disease progression, of conversion to inactive infection with low-level HBV DNA [3]. Diagnosing to HBV patients who are HBeAg negative, HBV DNA and ALT levels and detection of HBsAg can be used [3]. According to EASL guidelines, HBV DNA and ALT levels of the patients with HBeAg negative whose HBV DNA levels are under 2000 IU/ml should be determined every year and should be followed up in terms of fibrosis every 3 years [3].

Detection of anti-HBe; anti-HBe antibody is formed after HBeAg and HBsAg antibodies become nagtive. Anti-HBe seroconversion states decreasing in HBV replication, transformation of acute hepatitis B infection to chronic hepatitis B infection [11]. There is no detected role of anti-HBe antibody in destroying, preventing or controlling HBV infection [16].

Detection of anti-HBc; Anti-HBc antibodies are formed against core antibody of HBV. It is indicator of HBV infection. It can not be detected after the vaccination. HBc antibodies are not found in blood in high levels. Anti-HBc IgM antibody is the body's first response to acute Hepatitis B infection. It can be detected in the blood 6-8 weeks after contact to the patient. Anti-HBg IgM indicates that the infection occurred 6 months ago. After approximately 6-9 months, anti-HBc IgM level decreases, anti-HBc IgG level increases and can be detected in lifetime. Anti-HBc-IgM antibodies cannot generally be detected in chronic Hepatitis B infections [16].

Detection of HBV DNA level; Currently, diagnosing to acute and chronic HBV infection via detection of HBV DNA is accepted as the golden standard. Diagnosis to infection is based on the basis of the determining HBV DNA as qualitative and quantitative [3]. To diagnose to HBV infection, to decide on treatment options, to evaluate treatment response and to identify whether there is a complication risk of the infection, HBV DNA level needs to be detected and ALT level needs to be followed up [3, 4, 5]. Besides, HBV DNA is detected in determining patients with viremia but HBsAg negative [3]. Today, the amount of HBV DNA is determined with tests based on nucleic acid amplification with high specificity and sensitivity [3]. The World Health Organization enables comparison of different laboratory and test results by standardizing the calibration (international unit) of reagents used in HBV DNA nucleic acid amplification techniques with the IU unit [3].

It is decided to anti-HBV treatment by monitoring the levels of HBV DNA and ALT. To be started for anti-HBV treatment of the patient whose ALT level rises, recommended level of HBV DNA is minimum 2000 IU/ml for the patients with HBeAg negative and 20.000 IU/ml for the patients with HBeAg positive [4, 5].

HBsAg becomes positive in an average of 4 weeks (between 1-9 weeks) after it exposures to the agent in acute HBV infection [16]. Anti-HBc is a significant serum marker stating that HBV has been encountered. [3]. Besides, these patients are generally HBeAg positive [17].

Determination of HBV genotype; During the replication of HBV, due to high viral copy number and lack of proofreading activity of the reverse transcriptase enzyme, HBV genotypes, mutants and recombinants occur [18, 19, 20]. According to the phylogenetic analysis, there are 10 genotypes (A-J) and more than 30 subtypes of HBV [3]. These genotypes and subtypes have specific geographical distribution and transmission routes [21]. A, B and C genotypes are most common ones in the world. D genotype is mostly seen in our country [22]. Differences between genotypes do not affect treatment and screening of the patients [3].

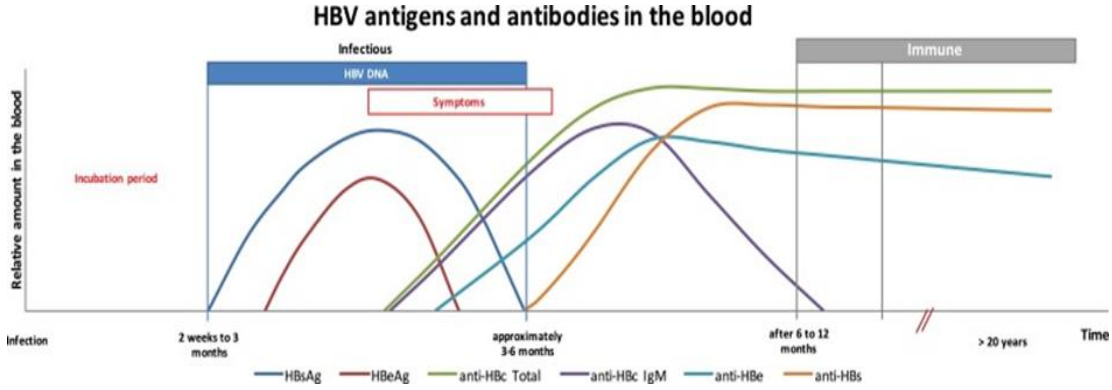

Fig. 1. *Antigens and antibodies profile in acute HBV infection. Bozza et al. 2016 [23].*

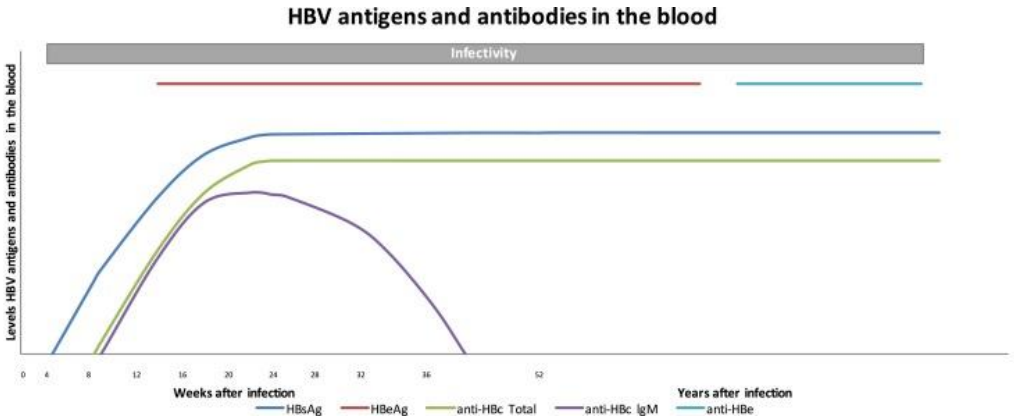

Fig. 2. *Antigens and antibodies profile in chronic HBV infection.Bozza et al. 2016 [23].*

Future Diagnostic Methods

Determination of ccc DNA; Intrahepatic ccc DNA is transcriptional template for HBV. ccc DNA enables reactivating HBV infection in anti-HBc positive people who are HBsAg negative but who use immunosuppressive drugs. Thus, relapses are formed after long-term nucleic acid therapy. cccDNA levels are high respectively in patients with HBeAg positive, HBeAg negative patients with immune active chronic HBV infection, inactive carriers and patients who have HBsAg loss. Southern blot analysis is used to detect cccDNA. However routine use of Southern blot analysis is complicated and it requires long time, besides it may not detect low levels. Therefore, it is not possible to use it as serum biomarker in the diagnosis of HBV infection [3].

Determination of serum HBV RNA level; it states HBV pregenomic (pg) RNA transcription activity copied from cccDNA [24]. HBV RNA level varies according to the phase of the infection. There is a connection between levels of HBV RNA and HBV DNA. The biomarker (with the levels of HBV DNA and HBsAg) can be used in the patients who receive nucleoside analog therapy for following-up the response of treatment; however, it needs to be supported by large-scale studies [3].

Determination of HBcrAg level; it is thought that HBcrAg which is an antigen related with HBV core is a marker of cccDNA and transcription. HBcrAg can be detected even in HBsAg negative patients. Thus, it can be used to develop therapeutic agents which target cccDNA. Treatment response will be able to be followed only by determining HbcrAg level or in addition to that by determining HbSAg and HBVDNA. Besides, therapeutic agents will able to be estimated [3].

Detection of HBeAg level; there exists positive correlation between levels of HBeAg and HBV DNA. Quantification of HBeAg which indicates viral replication can be used in monitoring disease progression, predicting HBeAg seroconversion and new anti-HBV treatments for following up the response of the treatment [3].

Detection of anti-HBc level; detection of anti-HBc level which is an antibody against HBV core protein antigen can be used as the marker of antiviral immune response or in detecting of the patients who are likely to respond to anti-HBV therapy [3].

II. CONLUSION

Currently, to diagnose HBV infection, to follow-up and manage patients, biomarkers expressing virus activity like HBsAg, HBeAg and HBV DNA are used. However, new diagnostic markers are needed to make the diagnosis and to better understand the pathogenesis of the infection, to monitor the progression of the disease and the treatment response, and to determine the activity of the virus and the immune response. For that reason, new tests need to be improved to detect anti-HBc, cccDNA, HBV RNA, HBeAg and HBcrAg and to identify levels of them.

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