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# Comparison of HBV-DNA Levels with Biochemical and Microbiological Parameters for Chronic Hepatitis Evaluation, Bursa, Turkey

**Sanem Karadag Gecgel**[1]

[1]Department of Microbiology and Clinical Microbiology, University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey

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***Correspondence**
**Email**: sanemkaradag@yahoo.com
**Tel**: +902243605050
**Fax**: +902243605055

ABSTRACT

**Introduction**: HBV-DNA levels are used to diagnose chronic hepatitis B (CHB) disease, determine the infection phase, decide on the treatment, and determine the disease course. We aimed to compare the microbiological and biochemical parameters of patients followed up with chronic hepatitis B pre-diagnosis in our hospital according to their HBV-DNA levels. **Methods**: HBV-DNA levels were analyzed using a Real time assay in blood samples of 500 pre-diagnosed CHB patients between February-June 2018, retrospectively. The biochemical parameters of the patients were measured by an automatic biochemical immunoassay analyzer (Beckman Coulter DXI 800, USA), and the microbiological parameters of patients were determined by an automatic analyzer (Roche Cobas 6000, Germany). The differences between the values of biochemical and microbiological parameters of patients were determined according to HBV-DNA. **Results**: Mean corpuscular volume (MCV) was higher in patients with hepatitis B virus-DNA (HBV-DNA)>20000 IU/mL than patients with negative HBV-DNA, HBV-DNA<2000 IU/mL, and HBV-DNA 2000-20000 IU/mL ($P$<0.05, $P$<0.01, $P$<0.01). Gamma-glutamyl transpeptidase (GGT) was lower in patients with HBV-DNA ranging from 2000-20000 IU/mL than HBV-DNA negative patients, HBV-DNA<2000 IU/mL and HBV-DNA>20000 IU/mL ($P$<0.05). Albumin was lower in patients with HBV-DNA>20000 IU/mL than patients with HBV-DNA 2000-20000 IU/mL and HBV-DNA<2000 IU/mL ($P$<0.01). Hepatitis B surface antigen (HBsAg) levels were higher in patients with HBV-DNA 2000-20000 IU/mL than HBV-DNA negative patients and patients with HBV-DNA<2000 IU/mL and HBV-DNA>20000 IU/mL ($P$<0.05, $P$<0.05, $P$<0.05). Albumin was individually correlated with the HBV-DNA by 2.9%, negatively ($P$<0.01). **Conclusion**: MCV, GGT, albumin, HBsAg levels might be used as indicators to diagnose CHB disease, establish the infection phase, decide the treatment, and determine the course of the disease together with HBV-DNA levels.

## INTRODUCTION

Worldwide, approximately 400 million people have chronic infection with hepatitis B virus (HBV) that might lead to different phases with outcomes ranging from asymptomatic carriers to cirrhosis, liver failure, and hepatocellular carcinoma (HCC) [1-3]. Several biomarkers correlated with hepatic diseases are used in clinical practice to monitor and predict disease progression. Hepatitis B-deoxyribonucleic acid (HBV-DNA) is a quantitative virologic indicator of HBV replication level. HBV-DNA levels are used to diagnose chronic hepatitis B (CHB) disease, determine the infection phase, decide on the treatment, and determine the disease course [4]. Previous studies showed that high serum HBV-DNA levels are a risk factor for liver damage and advanced liver diseases such as cirrhosis [5, 6]. Hepatitis B surface antigen (HBsAg) is the primary marker of HBV infection, and HBsAg clearance indicates the viral clearance. Hepatitis B e antigen (HBeAg) shows active viral replication and transcription and sign of infectivity. Alanine aminotransferase (ALT) is a marker of liver inflammation, levels of the upper limit of normal (ULN) are indicative of the injury to hepatocytes [7].

CHB infection is classified according to serum HBV-DNA level, ALT level, and HBeAg status; the infection process is evaluated by serial HBV-DNA and ALT

measurements [8-10]. According to recent studies, the HBsAg level can be a useful indicator in CHB patients management [11, 12]. In addition to HBsAg, HBeAg and HBV-DNA reache high levels during the initial CHB infection phase [13, 14]. High serum HBV-DNA and normal ALT levels are remarkable for HBeAg-positive patients in the immune tolerance (IT) phase. High HBV-DNA and high ALT levels are achieved after the IT phase later in the immune clearance (IC) phase in most patients [15]. The annual rate of HBeAg clearance range from 8%-12% in patients at the IC phase. HBeAg negativity and anti-HBe positivity, normal serum ALT concentration, low or undetectable HBV-DNA (<2000 IU/mL) are in patients with the no/low replicative phase (inactive chronic HBV carrier status) after HBeAg seroconversion. Positive HBsAg, negative HBeAg, usually positive anti-HBe, and persistent or fluctuating moderate to high HBV-DNA levels (often lower than in HBeAg-positive patients) and fluctuating or persistently elevated ALT levels are diagnostic for HBeAg-negative CHB [1, 4, 16]. Serial measurement of ALT and HBV-DNA levels are necessary for differentiating HBeAg-negative CHB from the inactive carrier state. The prognosis is good in the inactive carriage state, and the risk of progression to complications is lower than HBeAg negative CHB [4, 17]. HBeAg seroconversion may also be followed by HBsAg seroclearance, a state close to remission [18].

ALT>2 times ULN and HBV-DNA>20,000 IU/mL are the criteria for treating all HBeAg positive or HBeAg-negative chronic HBV patients with the immunoreactive phases [10, 14, 16]. HBV-DNA levels between 2000 and 20 000 IU/ml and normal ALT are defined as the ‘gray zone.’ The infection phase is determined by the follow-up period in this group of patients [1, 6]. Immediate liver biopsy or treatment is not required in HBeAg-negative CHB patients with normal ALT and HBV-DNA levels between 2000 and 20 000 IU/mL if they have no liver disease evidence. Nevertheless, it is recommended that they receive careful follow-up with serial ALT and HBV-DNA measurements [6]. Normal ALT and HBV- DNA <2,000 IU/mL represent an inactive carrier phase with an excellent long-term outcome with average overall life expectancy in HBeAg-negative patients. Biopsy and treatment are not required in inactive carriers, but lifelong follow-up is necessary with ALT and HBV-DNA determinations [6, 7, 14, 16].

Biochemical parameters [Aspartate aminotransferase (AST) and ALT, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), serum albumin, full blood count, and prothrombin time (PT)] are used for liver disease detection, the treatment decision, and the HCC surveillance [19]. Although HBV-DNA values and some biomarkers related to liver diseases are used in the diagnosis, classification, and treatment management of CHB, there are very few studies on other biomarkers that vary in different phases determining the clinical prognosis and characteristic of CHB.

We aimed to compare the microbiological and biochemical parameters of patients followed up with chronic hepatitis B pre-diagnosis in our hospital according to their HBV-DNA levels..

## MATERIALS AND METHODS

We performed a retrospective observational cohort study at the University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital, serving approximately 5 million people in Turkey's Southern Marmara region. We analyzed HBV-DNA, microbiological, and biochemical parameters' test results from blood samples of 500 patients referred to our hospital February-June 2018, retrospectively. We followed up patients at infectious diseases, gastroenterology, and children's outpatient clinics with pre-diagnosis of chronic viral hepatitis. We excluded from the study the patients diagnosed with acute viral hepatitis. We investigated the correlation of the HBV-DNA levels with biochemical parameters, including white blood cell (WBC), hemoglobin (HGB), mean corpuscular volume (MCV), red cell distribution width (RDW), alpha-fetoprotein, AST, ALT, ALP, GGT, albumin, PT, activated partial thromboplastin time (aPTT), international normalized ratio (INR). We also analyzed the correlation of the HBV-DNA levels with microbiological parameters as HBsAg, anti-HBs, HBeAg, and anti-HBe.

HBV-DNA was determined by isolation in the automatic device (Qiagen QIAsympony SP/AS Instrument, Germany) from the patients' plasma samples. The amplification of the obtained DNA was analyzed by Real-Time PCR in the automatic device (Qiagen Rotor-Gene Q (6000), Germany) simultaneously in our hospital's molecular microbiology laboratory. Biochemical parameters were measured by an automatic biochemical immunoassay analyzer (Beckman Coulter DXI 800, USA) in our hospital biochemistry laboratory. Microbiological parameters were determined by an automatic analyzer (Roche Cobas 6000, Germany) in our hospital's ELISA laboratory with the electrochemiluminescence method. HBV-DNA levels were divided into four groups: HBV-DNA negative, HBV-DNA<2000 IU /mL, HBV-DNA 2000-20000 IU /mL, and HBV-DNA>20000 IU /mL. The differences between the values of biochemical and microbiological parameters of patients according to HBV-DNA levels were determined.

The ethics committee of Yuksek Ihtisas Training and Research Hospital approved this study (ref: 2011-KAEK-25 2020/06-12).

**Statistical analysis**. Data were expressed by frequency or related percent values. The normality

data analysis (N>50) was done with the Kolmogorov-Smirnov test. Comparative analysis of the two groups was done with an independent sample T-test for normally distributed parameters and Mann-Whitney U test for non-normally distributed parameters. Comparative analysis of the more than two groups was done with one-way ANOVA and Welch tests for normally distributed parameters. Post Hoc LSD, Bonferroni, Scheffe, Tukey tests were used for the analysis of the difference. Comparative analysis of more than two groups was done with Kruskal-Wallis tests for non-normally distributed parameters. Statistical analysis of HBV-DNA test with microbiological tests was performed with the Chi-Square test. Correlation and regression analysis between the HBV-DNA test and laboratory tests with normal distribution was determined by the Pearson test. Correlation and regression analysis between the HBV-DNA tests and other laboratory tests with non-normally distribution was performed using the Spearman test. The analysis was done in the SPSS program. $P<0.05$ and $P<0.01$ were accepted as statistically significant.

## RESULTS

The biochemical and microbiological parameters of the CHB patients according to gender are shown in Table 1. WBC mean values ($P<0.05$) and HGB, MCV, GGT, albumin mean values ($P<0.01$) were significantly higher in male patients. RDW, AST, ALT, HBsAg mean values were significantly higher in female patients ($P<0.01$). There was no significant difference in age, HBV-DNA, alpha-fetoprotein, ALP, PT, aPTT, INR parameters according to the gender of the patients.

**Table 1.** Distribution of the variables by sex

| Variables | Sex | | *P* |
|---|---|---|---|
| | Female | Male | |
| | Mean± Std Deviation | Mean± Std Deviation | |
| Age | 45±14 | 43±14 | 0.121[a] |
| HBV-DNA | 35657±346515 | 174242±1498238 | 0.872[a] |
| WBC | 6.8±2.1 | 7.1±1.9 | 0.048[a] |
| HGB | 12.7±1.6 | 14.9±1.6 | 0.000[a] |
| MCV | 83.0±6.9 | 85.9±5.9 | 0.000[a] |
| RDW | 14.5±2.2 | 13.7±1.7 | 0.000[a] |
| α- feto protein | 8.9±33.4 | 8.0±66.4 | 0.535[a] |
| AST | 49±202 | 32±56 | 0.000[a] |
| ALT | 62±301 | 32±36 | 0.000[a] |
| ALP | 77±48 | 75±32 | 0.198[a] |
| GGT | 21±32 | 29±22 | 0.000[a] |
| Albumin | 4.58±0.47 | 4.73±0.57 | 0.004[b] |
| PT | 14.47±1.17 | 14.63±1.58 | 0.521[a] |
| aPTT | 29.71±5.69 | 29.71±2.89 | 0.431[a] |
| INR | 1.04±0.08 | 1.05±0.10 | 0.584[a] |
| HBsAg | 4003±2170 | 3423±2318 | 0.008[a] |

a: Mann Whitney U test b: Independent t test $P<0.01$, $P<0.05$

Comparison of biochemical parameters according to HBV-DNA levels is demonstrated in Table 2. HBV-DNA levels were categorized into four groups as HBV-DNA negative, HBV-DNA<2000 IU/mL, HBV-DNA 2000-20000 IU/mL, HBV-DNA> 20000 IU/mL.

MCV, AST, and ALT levels were found significantly higher in patients with HBV-DNA>20000 IU/mL than patients with HBV-DNA negative, HBV-DNA<2000 IU/mL and HBV-DNA 2000-20000 IU/mL ($P<0.05$, $P<0.01$, $P<0.01$).

GGT values were established significantly lower in patients with between HBV-DNA 2000-20000 IU/mL compared to the patients with HBV-DNA negative, HBV-DNA<2000 IU/mL, and HBV-DNA>20000 IU/mL ($P<0.05$).

Albumin levels were determined significantly lower in patients with HBV-DNA>20000 IU/mL compared to patients with HBV-DNA 2000-20000 IU/mL and HBV-DNA<2000 IU/mL ($P<0.01$). According to HBV-DNA levels, there was no statistically significant difference in WBC, HGB, RDW, PT, aPTT, INR, alpha-fetoprotein, and ALP values.

Comparison of HBsAg levels, according to HBV-DNA levels, is shown in Table 2. HBV-DNA values were categorized into four groups as HBV-DNA negative, HBV-DNA<2000 IU/mL, HBV-DNA 2000-20000 IU/mL, HBV-DNA>20000 IU/mL. HBsAg levels were assessed significantly lower in patients with HBV-DNA negative than patients with HBV-DNA<2000 IU/mL ($P<0.01$). HBsAg levels were found significantly higher in patients with HBV-DNA 2000-20000 IU/mL compared to patients with HBV-DNA negative, HBV-DNA<2000 IU/mL, and HBV-DNA>20000 IU/mL ($P<0.05$, $P<0.05$, $P<0.05$).

Comparison of microbiological parameters according to HBV-DNA levels is demonstrated in Table 3. HBV-DNA levels were categorized into four groups as HBV-DNA negative, HBV-DNA<2000 IU/mL, HBV-DNA 2000-20000 IU/mL, and HBV-DNA> 20000 IU/mL. HBsAg positivity was detected significantly lower in patients with HBV-DNA negative than patients

with HBV-DNA<2000 IU/mL and HBV-DNA 2000-20000 IU/mL ($P$<0.01). Anti-HBs positivity was assessed significantly higher in patients with HBV-DNA negative than patients with HBV-DNA<2000 IU /mL ($P$<0.01). HBeAg positivity was established significantly higher in patients with HBV-DNA>20000 IU/mL than patients with HBV-DNA<2000 IU/mL ($P$<0.05). Anti-HBe positivity was detected significantly higher in patients with HBV-DNA <2000 IU/mL compared to patients with HBV-DNA negative and HBV-DNA>20000 IU/mL ($P$<0.05, $P$<0.01).

**Table 2.** Comparison of biochemical parameters and HBsAg levels according to HBV-DNA levels

| Biochemical parameters | HBV-DNA Negative Mean±SD | <2000 Mean±SD | 2000-20000 Mean±SD | >20000 Mean±SD | *P* |
|---|---|---|---|---|---|
| WBC | 7.0±2.1 | 7.0±1.7 | 7.0±2.3 | 6.5±1.7 | 0.507[a] |
| HGB | 13.6±2.1 | 14.0±1.8 | 13.8±1.8 | 14.4±1.7 | 0.190[a] |
| MCV | 84.6±6.8 | 84.3±6.2 | 83.3±7.0 | 87.5±5.0 | 0.039[a], 0.044[c], 0.009[c], 0.003[c] |
| RDW | 14.2±1.9 | 14.0±2.0 | 14.1±2.0 | 14.4±3.1 | 0.617[a] |
| α-feto protein | 5.8±24.5 | 8.2±28.7 | 3.0±2.3 | 41.6±191.6 | 0.240[a] |
| AST | 35±95 | 23±7 | 24±6 | 225±525 | 0.000[a], 0.000[c], 0.000[c], 0.000[c] |
| ALT | 41±170 | 24±12 | 26±14 | 257±698 | 0.000[a], 0.000[c], 0.000[c], 0.000[c] |
| ALP | 80±45 | 71±26 | 65±21 | 102±78 | 0.077[a] |
| GGT | 25±19 | 23±13 | 20±15 | 56±93 | 0.033[a], 0.018[c],0.019[c], 0.014[c] |
| Albumin | 4.59±.0.55 | 4.74±0.47 | 4.76±0.42 | 4.41±0.64 | 0.003[b], 0.004[d], 0.006[d] |
| PT | 14.7±1.7 | 14.5±0.9 | 14.7±1.3 | 14.6±1.1 | 0.097[a] |
| aPTT | 30.1±5.6 | 29.2±2.8 | 29.9±2.5 | 29.4±1.9 | 0.445[a] |
| INR | 1.7±.01 | 1.0±0.1 | 1.1±0.1 | 1.1±0.1 | 0.116[a] |
| HBsAg | 3323±2444 | 3879±2116 | 4696±1677 | 3817±1865 | 0.001[a], 0,018[c], 0,000[c], 0,022[c], 0.034[c] |

a: Kruskal Wallis test b: One way ANOVA test c: Mann-Whitney U test d: Post Hoc LSD test $P$<0.01, $P$<0.05, SD: standard deviation

**Table 3.** Comparison of microbiological parameters according to HBV-DNA levels

| Microbiological parameters | HBV-DNA Negative n (%) | <2000 n (%) | 2000-20000 n (%) | >20000 n (%) | Negative n (%) | *P* |
|---|---|---|---|---|---|---|
| HBsAg | Negative | 28 (12,6) | 0 (0) | 0 (0) | 0 (0) | 0.000[a] |
| | Positive | 194 (87,4) | 157(100) | 57 (100) | 27 (100) | 0.002[a] |
| Anti-HBs | Negative | 190 (89,6) | 148 (97,4) | 52 (98,1) | 25 (96,2) | 0.006[a] |
| | Positive | 22 (10,4) | 4 (2,6) | 1 (1,9) | 1 (3,8) | |
| HBeAg | Negative | 74 (90,2) | 50 (96,2) | 24 (92,3) | 9 (69,2) | 0.012[a] |
| | Positive | 8 (9,8) | 2 (3,8) | 2 (7,7) | 4 (30,8) | |
| Anti-HBe | Negative | 15 (17,9) | 2 (3,6) | 2 (7,7) | 4 (33,3) | 0.015[a] |
| | Positive | 69 (82,1) | 54 (96,4) | 24 (92,3) | 8 (66,7) | 0.007[a] |

a: Chi-square test $P$<0.01, $P$<0.05

Correlation and regression analysis between the HBV-DNA and biochemical parameters is demonstrated in Table 4. The HBV-DNA levels were correlated positively with ALT and negatively with albumin ($P$ <0.01, $P$<0.01). There was no significant correlation between HBV-DNA values and WBC, HGB, MCV, RDW, alpha-fetoprotein, AST, ALP, GGT, PT, aPTT, INR values.

Single regression analysis of biochemical parameters affecting HBV-DNA values is revealed that ALT was individually correlated with the HBV-DNA by 4.0% positively ($P$<0.01). Albumin was individually correlated with the HBV-DNA by 2.9% negatively ($P$<0.01).

## DISCUSSION

The liver disease development and progression are associated with systemic inflammatory responses. Inflammatory and hematological biomarkers levels define the severity of liver disease [20]. Several hematological markers, especially RDW, are essential indicators of adverse outcomes in HBV-related liver diseases [21, 22]. In our study, hematological markers, WBC, HGB, and RDW values did not change according to HBV-DNA values, while MCV values were significantly higher in patients with HBV-DNA>20000 IU/mL compared to patients with lower HBV-DNA values ($P$<0.05). MCV, a measurement of the average volume of red blood cells, increases in many clinical conditions [23, 24]. Nevertheless, there are few studies about the clinical usefulness of MCV in liver disease. Yang *et al*. (2018) reported that the MCV was associated with the severity of liver impairment and could be a predictor for mortality in patients with HBV- related decompensated cirrhosis [23]. A study demonstrated that MCV values were significantly higher in patients with chronic liver failure than healthy controls [21]. In our study, significantly higher MCV levels and higher ALT and AST levels in patients with HBV-DNA>20000 IU/mL show that the MCV can be used as a non-invasive parameter in the diagnosis and treatment evaluation together with ALT and HBV-DNA. However, more studies are needed, including advanced examination, follow-up, and treatment.

**Table 4.** Correlation analysis between the HBV-DNA and biochemical parameters

| Test | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HBV-DNA** | 1[a, b] | | | | | | | | | | | | |
| **WBC** | -,043[a] | 1[a] | | | | | | | | | | | |
| **HGB** | ,077[a] | ,156[a**] | 1[a] | | | | | | | | | | |
| **MCV** | -,004[a] | ,029[a] | ,389*[a**] | 1[a] | | | | | | | | | |
| **RDW** | -,030[a] | ,024[a] | -,510[a**] | -,483[a**] | 1[a] | | | | | | | | |
| **α- feto protein** | ,075[a] | -,016[a] | ,040[a] | ,039[a] | ,021[a] | 1[a] | | | | | | | |
| **AST** | ,092[a] | -,086[a] | ,142[a**] | ,146[a**] | -,049[a] | ,100[a*] | 1[a] | | | | | | |
| **ALT** | ,187[a**] | ,055[a] | ,373[a**] | ,194[a**] | -,213[a**] | ,087[a] | ,751[a**] | 1[a] | | | | | |
| **ALP** | -,065[a] | ,196[a**] | ,124[a*] | -,119[a*] | ,024[a] | -,004[a] | ,204[a**] | ,172[a**] | 1[a] | | | | |
| **GGT** | -,049[a] | ,151[a**] | ,434[a**] | ,173[a**] | -,138[a**] | ,056[a] | ,319[a**] | ,494[a**] | ,226[a**] | 1[a] | | | |
| **PT** | -,064[a] | -,179[a**] | -,098[a] | ,008[a] | ,143[a**] | -,012[a] | ,098[a] | -,032[a] | -,069[a] | -,046[a] | 1[a] | | |
| **aPTT** | -,005[a] | -,064[a] | ,030[a] | ,100[a] | ,008[a] | ,062[a] | ,011[a] | -,049[a] | -,134[a*] | -,078[a] | ,252[a**] | 1[a] | |
| **INR** | -,067[a] | -,172[a**] | -,099[a] | ,005[a] | ,141[a**] | -,011[a] | ,088[a] | -,042[a] | -,067[a] | -,049[a] | ,998[a**] | ,254[a**] | 1[a] |
| **Albumin** | -,171[b**] | 1[b] | | | | | | | | | | | |

a: Spearman test value, b: Pearson test value** $P<0.01$ * $P<0.05$

Albumin is an acute-phase protein synthesized from the liver—the synthesis and functions of albumin decay in patients with liver failure [25, 26]. Albumin values decrease significantly in chronic liver diseases such as cirrhosis with liver damage and indicate prognosis [27, 28]. High albumin level reflects better nutrition status, immune response, and liver function and demonstrates better liver disease outcomes [29]. A study showed that a lower albumin level or a higher AST or ALT level are associated with a higher hepatitis B viral load in liver diseases [30]. Nakamuta *et al*. (2007) demonstrated that albumin levels were associated with HBV-DNA levels but not with ALT levels [31]. In our study, albumin was individually correlated with the HBV-DNA by 2.9% negatively ($P<0.01$), and albumin was determined as the biomarker that most affected HBV-DNA after ALT. Also, significantly lower albumin levels in patients with HBV-DNA>20000 IU/mL than patients with HBV-DNA<20000 IU/mL ($P<0.01$) suggests that albumin levels may also be used as the treatment criterion for patients with HBV-DNA>20000 IU/mL.

Serum GGT level is an indicator of hepatobiliary diseases, alcohol intake. Serum GGT has been accepted as a potential biomarker for diagnosing and treating HBV infection [32]. Several studies showed that high serum GGT levels are achieved in severe liver inflammation in CHB, liver cirrhosis, advanced tumors, and adverse outcomes [33-35]. GGT and albumin levels were used to predict the short-term outcomes of hepatitis B virus, associated liver failure, and the management of artificial liver support therapy during the early stage [36]. According to a study, the presence of HBV-DNA in maternal blood is significantly associated with maternal serum GGT levels during the third trimester of pregnancy [37]. High serum GGT levels are found in HBeAg positive and negative CHB patients and can determine the activated antiviral immune responses and HBeAg seroconversion after antiviral therapy [32]. A significant decrease in GGT, ALT, and AST values was detected in a study, along with decreased HBV-DNA values in patients with CHB [38]. Our study showed a significant decrease in GGT values between HBV-DNA 2000-20000 IU/mL ($P<0.05$), but no correlation was between GGT values and HBV-DNA values. Our study showed that on assessing treatment decisions with the HBV- DNA values (2000-20000 IU/mL) for the patients requiring advanced invasive examination, GGT values may guide the necessity of treatment, response treatment and liver injury.

Several studies showed that a low and undetectable serum HBV-DNA and HBsAg level were significant indicators of good long-term prognosis in chronic HBV infection and were detected more in inactive HBV carriers than patients with chronic active hepatitis B [39, 40]. According to some studies, HBsAg levels can predict treatment response in CHB [41, 42]. Like previous reports, in our study, HBsAg values were significantly lower in negative HBV-DNA patients, defined as inactive carrier chronic hepatitis B patients, compared to patients with low HBV-DNA values, regarded as CHB patients ($P<0.01$). According to our study, HBsAg values in chronic hepatitis B disease indicate the establishment of negative HBV-DNA levels, that is, in the transition to the inactive carrier phase. Also, significantly higher HBsAg values in patients with HBV-DNA between 2000-20000 IU/mL ($P<0.05$) show that HBsAg values can be used alongside HBV-DNA to indicate treatment necessity and to determine treatment response in patients with HBV-DNA values requiring advanced invasive examination.

According to a study, HBV-DNA levels were higher in HBeAg-positive CHB patients compared to HBeAg-negative CHB patients [4]. HBeAg seroconversion with undetectable HBV-DNA levels is an essential aim in the treatment management of CHB patients [43, 44]. HBeAg seroconversion may proceed to the inactive carrier phase characterized by HBeAg negativity, anti-HBe positivity, and low or undetectable HBV-DNA [45]. In our study, a statistically significant higher HBeAg positivity in patients with HBV-DNA>20000 IU/mL ($P<0.05$) compared to patients with HBV-DNA<2000 IU/mL shows the treatment necessity of CHB patients with HBV-DNA>20000 IU/mL. In our study, significantly higher detection of Anti-HBe positivity in patients with HBV-DNA<2000 IU/mL compared to the patients with HBV-DNA negative and HBV-DNA>20000 IU/mL ($P<0.05$, $P<0.01$) indicates much more transition to the inactive carrier phase in CHB patients with HBV-DNA<2000 IU/mL.

High serum ALT and AST levels are assumed to be specific for liver damage, as they are in low concentration in tissues outside the liver. ALT level is commonly used to identify patients who need treatment. However, various factors can influence ALT, and this enzyme is not always well correlated with liver inflammatory activity grade, and some patients with normal ALT levels have significant inflammation or fibrosis [1, 46]. According to treatment guidelines, CHB patients with persistently normal ALT levels should not get antiviral therapy except for liver cirrhosis patients and patients with liver biopsies that exhibit significant fibrosis. [47, 48]. HBV-DNA>20,000 IU/ml and ALT>2 times ULN levels are the treatment criteria even without a liver biopsy for CHB patients [4]. ALT is more sensitive to the decrease in HBV-DNA viral load than other liver markers in CHB patients [49]. According to our study, a 4% positive correlation ($P$<0.01) was between HBV- DNA values and ALT values, showing that the HBV-DNA values are positively affected by the ALT values. According to our study, higher ALT and AST values were detected in CHB patients with HBV-DNA>20000 IU/mL ($P$<0.01, $P$<0.01). Hence, antiviral therapy treatment is more required in CHB patients with HBV-DNA>20000 IU/mL.

MCV, albumin, GGT, HBsAg levels, HBeAg, and anti-HBe status and ALT levels can be used to diagnose CHB disease and establish the infection phase, decide the treatment and determine the course of the disease together with HBV-DNA levels.

Our study limitation was the lack of HBV genotyping, as it is not performed routinely in clinical practice. For decreasing invasive methods in CHB evaluation, further studies should examine other non-invasive parameters.

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