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# Investigation of the association of serum quantitative HBsAg levels with viral load in chronic hepatitis B Patients receiving treatment

## *Tedavi alan kronik hepatit B hastalarında serum kantitatif HBsAg düzeyi ile viral yük arasındaki ilişkinin araştırılması*

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**Abstract**

**Purpose:** The Hepatitis B surface antigen (HBsAg) test was only used for diagnosis of hepatitis B infection, whereas now there is growing evidence favoring utilization of serum HBsAg quantitation (qHBsAg) for monitoring of infection with hepatitis B virus (HBV). In our study, we examined the courses of qHBsAg and HBV-DNA levels throughout the treatment course of patients receiving treatment for chronic hepatitis B infection and evaluated their correlation with each other.

**Material and methods:** The study was performed Ankara Training and Research Hospital. A total of 71 patients receiving treatment for the diagnosis of chronic hepatitis B (CHB) infection were included in the study. Serum qHBsAg levels of these patients were analyzed with an Elecsys HBsAg II assay.

**Results:** When we evaluated the correlation of serum qHBsAg levels with the HBV-DNA levels among patients treated with nucleotide analogues, there was a positive correlation. The decline in both parameters at sixth and 12-month follow-up was found to be statistically significant (qHBsAg decline at 6th month: $p$=0.03; qHBsAg decline at 12th month: $p$=0.001; HBV-DNA decline at 6th month: $p$=0.001; HBV-DNA decline at12th month: $p$=0.001). In the group of patients receiving PEG-IFN treatment, there was positive correlation between the two at the end of the treatment ($p$=0.01).

**Conclusion: :** Our findings suggest that qHBsAg can be a very useful test when hbv-DNA levels are used together to separate inactive HBsAg carriers and KHB patients and may be used for both the treatment decision and monitoring of treatment.

**Key words:** Chronic Hepatitis B, inactive HBsAg carrier, qHBsAg, PEG-IFN, nucleotide analogues.

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**Özet**

**Amaç:** Yakın geçmişe kadar HBsAg sadece hepatit B enfeksiyonun tanısında kullanılırken, günümüzde serum HBsAg kantitasyonu (qHBsAg)'nun, hepatit B virüsü (HBV) enfeksiyonunun viral monitörizasyonunda kullanılabileceğine dair veriler artmaya başlamıştır. Bu çalışmada inaktif hepatit B taşıyıcıları ile kronik aktif hepatit B hastaları arasındaki qHBsAg ile HBV DNA düzeyleri karşılaştırılmış ve tedavi başlanmış hastalarda tedavi süresince qHBsAg ile HBVDNA seyri izlenerek aralarındaki ilişki değerlendirilmiştir.

**Gereç ve yöntem:** Çalışma Ankara Eğitim ve Araştırma Hastanesi Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği'nde yapılmıştır. Kronik hepatit B (KHB) tanısıyla tedavi alan 71 hasta ve inaktif HBsAg taşıyıcısı 74 hasta çalışmaya dahil edildi. Bu hastalarda serum qHBsAg düzeyleri Elecsys HBsAg II cihazı ile çalışıldı.

**Bulgular:** Nükleoz(t)id analoğu ile tedavi edilen hastalarda 6. ve 12. aydaki serum qHBsAg düzeyi ile HBV-DNA arasında pozitif yönde bir korelasyon görüldü ve her iki parametrenin altı ay ve bir yıllık izlemindeki düşüş istatiksel olarak anlamlı bulundu (qHBsAg 6ay $p$=0,03, qHBsAg 12 ay $p$=0,001, HBV DNA 6 ay $p$=0,001, HBV DNA 12 ay $p$=0,001). Peg-IFN tedavisi alan kronik hepatit B hastalarında ise serum qHBsAg düzeyi ile HBV-DNA arasında tedavi başlangıcı ve 6. ayında anlamlı bir ilişki olmayıp tedavi sonunda pozitif yönde korelasyon görüldü. PEG-IFN tedavisi alan hasta grubunda ise qHBsAg seviyeleri ile HBV DNA arasındaki korelasyon tedavi sonunda anlamlı ($p$=0,01) olarak saptandı.

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**Sonuç:** Bizim bulgularımız qHBsAg'nin, inaktif HBsAg taşıyıcıları ve KHB hastalarını ayırmada HBV-DNA düzeyleri birlikte kullanıldığında oldukça faydalı bir test olabileceği ve tedavi kararında ve takibinde qHBSAg seviyelerinin kullanılabileceğini düşündürmektedir.

**Anahtar kelimeler:** Kronik Hepatit B, inaktif HBsAg taşıyıcılığı, qHBsAg, PEG-IFN, nükleoz(t)id analogları.

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## Introduction

It is estimated that approximately two billion people worldwide have encountered the hepatitis B virus. In addition, approximately 250 million people carry the virus, and hundred thousand people die each year due to either hepatitis B infection or hepatitis B related end stage liver disease [1].

The purpose of the treatment in chronic HBV infection is the suppression of viral replication, remission of liver disease, and clinical healing [2]. The gold standard method for monitoring viral load is by examination of viral RNA levels by polymerase chain reaction (PCR) [3]. Its lack of availability and high cost are some disadvantages of PCR in some centers. Currently, *Hepatitis B surface Antigen* (HBsAg) levels can be easily measured with a quantitative method in every center. Therefore, studies have focused on the use of serum HBsAg level as a marker of viral load instead of HBV-DNA. In chronic hepatitis B, the serum HBsAg level is known to be associated with the amount of covalently closed circular DNA (cccDNA) of HBV in the liver. Some studies suggest a potential new role for HBsAg quantitation in the response evaluation to viral treatment, especially with Pegylated Interferon (PEG-IFN). It seems quantitation of HBsAg is beneficial for evaluation of early treatment response at the 12th to 24th week of treatment, and for the determination of treatment duration [4-6]. The guideline of the European Association for the Study of Liver (EASL) recommends the measurement of HBsAg levels for monitoring response in patients receiving interferon treatment [7].

In this study, we measured HBsAg levels and investigated its correlation with HBV-DNA levels at the initiation of treatment and during a one-year follow-up period of patients with chronic HBV infection receiving oral antiviral and interferon treatment. Thus, we aimed to determine the significance of HBsAg level for diagnosis and for monitoring of treatment in patients with chronic hepatitis B.

## Material and methods

The study included patients diagnosed with chronic active hepatitis B between 01/01/2012 to 01/06/2013 by the Infectious Diseases and Clinical Microbiology Clinic of Ankara Training and Research Hospital. The study was approved by our hospital's Training Planning Coordination and Ethic Committee with an issue date of 16.01.2013 and issue number 4065.

Patient selection and tests: Active chronic hepatitis B was defined as the presence of HBsAg positivity for at least six months together with normal or elevated ALT values, positive or negative HBeAg results, HBV-DNA levels ≥2000 I.U, and presence of chronic hepatitis signs in histopathological examination. These patients were taken into treatment in accordance with the rules of the Health Application Declaration of Ministry of Health in Turkey [8]. Patients who had delta or another coinfection were excluded from the study. Blood samples were obtained from patients at the time of initiation, at 6$^{th}$, and 12th months of treatment. Following centrifugation at 3000-5000 rpm for 10-15 minutes, serum samples were stored at –80°C until the time of qHBsAg analysis. For all patients, HBsAg, HBeAg, and Anti-HBe tests were performed in our hospital's Microbiology Laboratory using an ELISA (micro-ELISA-Radim/Alisei, Italy; macro-ELISA-Vitros ECI, Ortochemical Diagnostics, USA) method. The HBV-DNA tests were performed using quantitative PCR method (Abbott real time HBV).

Quantitation of HBsAg: qHBsAg was measured with an Elecsys HBsAg (Roche Diagnostics) commercial kits. The test principle was an electro-chemiluminescence immunoassay. The test was performed with serum samples that were diluted 100 times automatically with a Cobas e411 analyzer.

Detection of the limit for this test was between 5 to 13,000 IU/ml. Samples that had HBsAg levels above 13,000 IU/ml were analyzed according to the assay protocol by diluting with the HBsAg diluent in a 1:10 ratio.

### 3.3. Statistical analysis

Statistical analysis was performed on SPSS 16.0 for Windows software. Independent group comparison was made between HBeAg-positive and HBeAg-negative patients. For numerical variables, a t-test was used for normally distributed data, and the Mann-Whitney U-test and Wilcoxon Signed Ranks test were used for non-normally distributed data. For patient groups receiving treatment, the correlation between HBV-DNA and qHBsAg was analyzed with Pearson and Spearman correlation coefficients.

A p-value of less that 0.05 was accepted as significant.

## Results

The study included a total of 71 patients with chronic hepatitis B infection receiving treatment. Demographical properties and initial laboratory results are given in Table 1.

When patients receiving nucleotide analogues were evaluated, 31 patients were receiving lamivudine and 23 patients were receiving tenofovir. Since the number of patients receiving telbivudine and entecavir were low, they were evaluated among patients receiving general antiviral drugs. There were 15 (21.1%) HBeAg-positive patients. Of these 15 (21.1%) patients, 8 were receiving tenofovir, 4 were receiving Peg-IFN, and 3 were receiving lamivudine treatment. The correlation between qHBsAg and HBV-DNA was analyzed separately in the patient groups receiving tenofovir and lamivudine treatment. The correlation between qHBsAg and HBV-DNA was analyzed during the follow-up of patients receiving all nucleotide

**Table 1.** Demographical and laboratory features of patients with chronic Hepatitis B infection

| **Patients with chronic hepatitis B infection (n=71)** | |
|---|---|
| Age ± SD | 40.08±11.7 |
| Sex (F/M) % | 47.8/52.1 |
| White blood cells, x10³/µl ± SD | 6683.8±1315.1 |
| Hemoglobin, g/dl ± SD | 14.2±1.9 |
| Thrombocyte, ×10³/µL± SD | 221088±50977. |
| ALT, U/L ± SD | 53.7±54.02 |
| AST, U/L ± SD | 39.6±36.7 |
| ALP, U/L ± SD | 82.3**±25.3** |
| GGT, U/L ± SD | 28.1±21.4 |
| LDH, U/L ± SD | 170.3±18.1 |
| AFP, IU/ml ± SD | 3.04±2.7 |
| HBeAg-positive patients % | 15 (21.1) |
| HBeAg-negative patients % | 56 (78.9) |
| Patients receiving Peg-IFN % | 11 (15.5) |
| Patients receiving nucleotide analogue % | 60 (84.5) |

analogues and pegylated interferon treatment. In addition, qHBsAg levels were measured among HBeAg-positive and HBeAg-negative patients at the time of initiation, 6th, and 12th months of treatment.

For patients receiving lamivudine, the mean initial HBV-DNA value was 25.677.2±101.316.4 IU/ml, HBV-DNA value at 6th month was 185.3±607.5, and HBV-DNA value at 12th month was 249.8±885.1 IU/ml. When compared to the initial values, the decline in HBV-DNA levels was significant both at the 6th month and at the end of one year ($p$=0.001), whereas the difference between the 6th and 12th month values was not statistically significant ($p$=0.44). The qHBsAg value was initially 6.774±9.795.8 IU/ml; 6.156.8±9.116.9 IU/ml at the 6th month; and 5.764.2±9.113.3 IU/ml at the 12th month. The decline in qHBsAg levels was statistically significant for the first 6 months ($p$=0.05), between 6 to 12 months ($p$=0.04), and during the one-year follow-up period ($p$=0.002). When the correlation between the initial qHBsAg and HBV-DNA values was evaluated in the group receiving lamivudine treatment, there was no significant correlation between them. The courses of HBV-DNA and qHBsAg at the 6th and 12th months are shown in Figure 1.

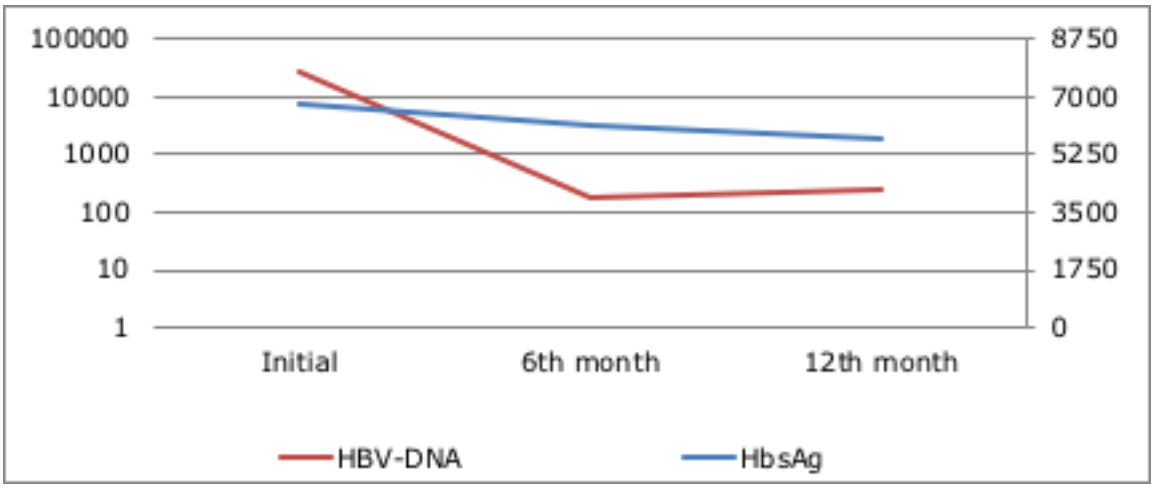


**Figure 1.** Courses of qHBsAg and HBV-DNA during the course of treatment among patients receiving lamivudine.

Twenty-three patients received tenofovir treatment. The mean initial HBV-DNA value was 10.000.000±137.670.685 IU/ml, the mean HBV-DNA value at 6th month was 9.685.7±23.611.6, and the mean HBV-DNA value at 12th month was 768±1974.51 IU/ml. When HBV-DNA levels were compared to initial values, there were significant reductions both at the 6th month ($p$=0.001) and at the 12th month ($p$=0.003). The reduction in HBV-DNA levels between the 6th and 12th month was also significant ($p$=0.012). The initial qHBsAg value was 50.928±89.098 IU/ml, the qHBsAg value at the 6th month was 35.833±59.297.3 IU/ml, and the qHBsAg at the 12th month was 13.477.3±13.495.8 IU/ml. The reduction in qHBsAg level was not found to be statistically significant during the first six months ($p$=0.14), whereas there was significant reduction in the qHBsAg level both between 6th to 12th month and ath the one-year follow-up ($p$=0.019). For the patient group receiving tenofovir, there was a significant correlation between qHBsAg and HBV-DNA levels. The courses of HBV-DNA and qHBsAg at the 6th and 12th month are shown in Figure 2.

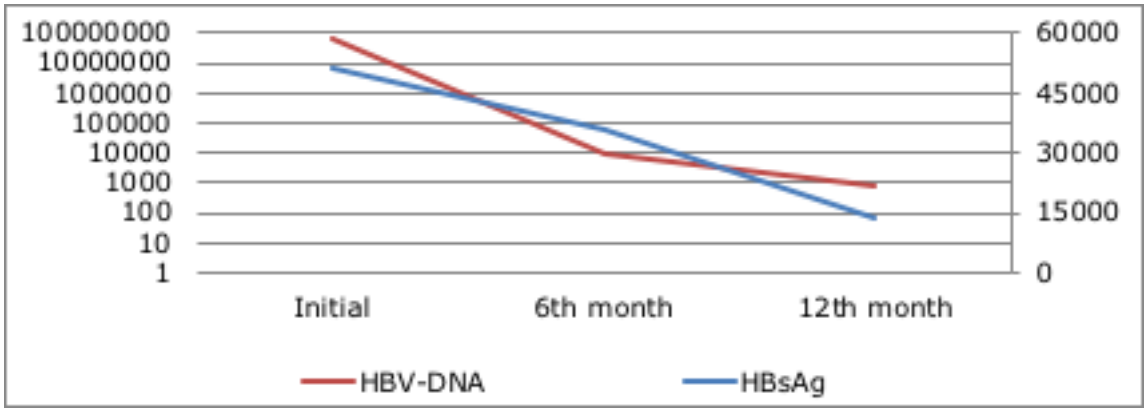


**Figure 2.** Courses of qHBsAg and HBV-DNA during the course of treatment among patients receiving tenofovir

A total of 60 patients were treated with nucleotide analogues. The mean initial HBV-DNA value among these patients was 30.000.000±90.000.000 IU/ml, HBV-DNA value at the 6th month was 3.605.9±14.736.4 IU/ml, and the mean HBV-DNA value at the 12th month was 373.5±1.261.1 IU/ml. When HBV-DNA levels were compared to the initial values, the decline was statistically significant during the first six months ($p$=0.001), between 6th to 12th months ($p$=0.017), and at the one-year follow-up ($p$=0.001). The initial qHBsAg value was 23.543.7±59.096.2 IU/ml, the qHBsAg value at the 6th month was 17.561.5±39.761.1 IU/ml, and the qHBsAg value at the 12th month was 8.744.6±12.052.2 IU/ml. The qHBsAg titers showed a significant reduction during the first six months ($p$=0.03), between 6th to 12th months ($p$=0.005), and at the one-year follow-up time ($p$=0.001). When the correlation between qHBsAg and HBV-DNA levels was evaluated in patients with chronic hepatitis B receiving nucleotide analogues, there was a positive correlation between them. The reduction in both qHBsAg and HBV-DNA levels at 6th and 12th months was in similar proportions. Their courses are shown in Figure 3.

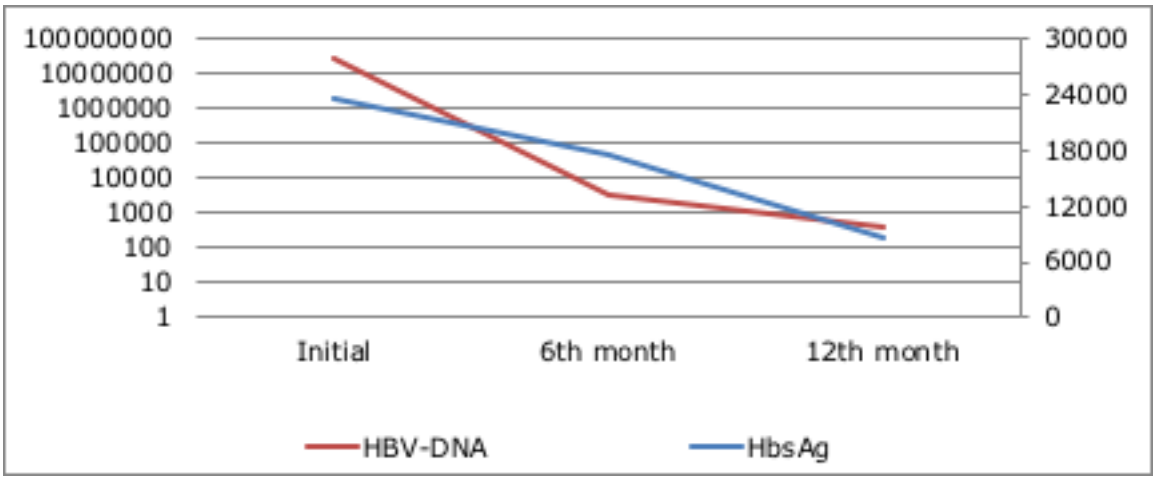


**Figure 3.** Courses of qHBsAg and HBV-DNA during the course of treatment among patients receiving nucleos(t)ide analogues

Eleven patients received PEG-IFN treatment. The mean initial HBV-DNA value among these patients was 200.000.000±400.000.000 IU/ml, the HBV-DNA value at the 6th month was 19.221.6±34.364.7, and the mean HBV-DNA value at the 12th month was 5.621±11.039.2. When compared to the initial values, the decline in HBV-DNA titers during follow-up was significant at both the 6th month ($p$=0.005) and 12th month ($p$=0.01). However, the difference between 6th and 12th months was not statistically significant ($p$=0.08). The mean initial qHBsAg level was 56.160.8±88.683.8 IU/ml, the qHBsAg level at 6th month was 41.376.3±58.713.8 IU/ml, and the qHBsAg level at the 12th month was 24.509.1±49.331.2 IU/ml. When compared to the initial values, it was significant for both the one-year follow-up and the 6th to 12th months intervals ($p$=0.01); although, the decline in qHBsAg level was not statistically significant during the first six months ($p$=0.65).

When the correlation between the qHBsAg and HBV-DNA levels was evaluated among patients with chronic hepatitis B treated with PEG-IFN, there was no significant correlation both at the time of initiation and the 6th month of treatment. However, there was a positive correlation in comparison of values at the end of treatment.

**Discussion**

The purpose of treatment in chronic HBV infection is to suppress viral replication (reduction in serum HBV-DNA to undetectable levels, HBeAg/anti-HBe seroconversion, HBsAg/anti-HBsAg seroconversion), reduce liver disease (normalization of serum ALT levels, decreased necroinflamation in liver), and promote clinical healing (reduced risk of development of cirrhosis, liver failure and hepatocellular carcinoma, increased life expectancy).

Although HBV replication does not have a direct cytopathic effect, study results indicate an association between ongoing viral replication and the degree of liver injury. Since viral elimination cannot be fully achieved with current treatment modalities, the target in anti-viral treatment is long-term viral suppression.

Results of large-scale cohort studies show a close association between high levels of HBV-DNA and hepatocellular carcinoma. The main target of anti-viral treatment is to reduce HBV-DNA to undetectable levels [2]. The gold standard method for monitoring viral load is polymerase chain reaction (PCR) [3], and the main disadvantage of this methods is its lack of availability in some centers, and its high cost. On the other hand, there are studies that suggest measurement of HBV-DNA level alone is not sufficient for the prevention of hepatocellular carcinoma development [9-11]. Since HBsAg levels can easily be measured quantitatively in every center, studies have focused on whether serum HBsAg level could be used instead of HBV-DNA. In addition, the correlation of qHBsAg with HBV-DNA level and cccDNA has become of interest.

In our study, we examined the correlation between qHBsAg and HBV-DNA levels for assessment of treatment response both in patients receiving PEG-IFN and patients receiving nucleos(t)ide analogues. Since the number of patients receiving telbivudine and entecavir treatment in our study was low, they were evaluated among the patients receiving general antiviral drugs. Additionally, HBsAg titer in patients receiving lamivudine and tenofovir was evaluated separately.

Lamivudine treatment was administered in 31 patients, and all were in the HBeAg-negative group. According to Health Application Declaration rules that are in effect in Turkey, lamivudine treatment, when compared to other treatment modalities, is initiated at lower HBV-DNA levels. Therefore, HBV-DNA levels, as well as HBsAg titers were low in this group. When compared to the initial values, the decline in HBV-DNA level was significant both at the 6th month of treatment and at the end of a one-year follow-up ($p$=0.001). qHBsAg levels were initially 6.773.9±9.795.8 IU/ml, 6.156.8±9116.8 IU/ml at 6th month, and 5.764.2±9.113.2 IU/ml at 12th month. When compared to initial values, the decline in qHBsAg levels was statistically significant both at 6th month ($p$=0.05), and at the end of one-year follow-up ($p$=0.002). In our study, it was observed that the decline in HBV-DNA was more rapid during the first months, and HBsAg titers declined later. Previous studies have shown that greater than 1 log rapid decline in HBsAg titer in patients receiving lamivudine is associated with permanent viral loss. Long-term

follow-up of patients will be informative in this regard.

In our study, 23 patients received tenofovir treatment. When compared to initial values, there was significant decline in HBV-DNA levels both at 6th month ($p$=0.001) and at 12th month ($p$=0.003). As for qHBsAg levels, while there was no significant decline during the first six months ($p$=0.14), there was significant decline at the end of one-year follow-up ($p$=0.019). In their study, Gane et al. [12] followed up patients receiving tenofovir for nearly 3 years, and they found that the decline in HBsAg level was more prominent among HBeAg-positive patients. Among these HBeAg-positive patients, they found the proportion of patients who lost HBsAg at first, second, and third year of treatment was 3%, 6% and 8%, respectively. They did not detect HBsAg loss among HBeAg-negative patients. Higher decline in HBsAg was associated with a higher chance for spontaneous HBsAg loss. In another study, a rapid fall in HBsAg during the first year of treatment with tenofovir was more closely associated with HBsAg loss [13]. In our study, we could not interpret HBsAg loss, since our follow-up time was limited to one year.

Previous studies suggest that HBsAg levels decline slowly during treatment with nucleotide analogues [12, 14]. In their study involving patients treated with various nucleotide analogues, Chevaliez et al. [15] noted a slow but consistent decline in HBsAg levels among 27 of 30 patients undergoing treatment. It has been estimated that it takes approximately 35 years to get rid of HBsAg in HBeAg positive patients [16]. In fact, it is known that nucleotide analogues only block reverse transcriptase, and that HBV-DNA synthesis is decreased without a direct effect on cccDNA. Although the mechanism of the decline in HBsAg during treatment with nucleotide analogues is not fully clear, restoration of host immune response, a better immune control against the virus, or a reduction in the amount of cccDNA might be assumed to cause the reduction in HBsAg levels [17].

In our study, 60 patients were treated with nucleotide analogues. Among these patients, initial mean HBV-DNA level was 30.000.000±90.000.000 IU/ml, HBV-DNA level at 6th month was 3.605.9±14.736.4 IU/ml and HBV-DNA level at 12th month was 373.5±1.261.1 IU/ml. When compared to initial values, the decline in HBV-DNA titers was statistically significant both at 6th month ($p$=0.001) and at 12th month ($p$=0.001). Initial qHBsAg level was 23.543.7±59.096.2 IU/ml, and qHBsAg levels were 17.561.5±39.761.1 IU/ml at 6th month and 8.744.6±12.052.2 IU/ml at 12th month. qHBsAg titers showed significant decline both at the 6th month ($p$=0.03) and at the end of one-year follow-up ($p$=0.001) when compared to initial values. Among patients with chronic hepatitis B treated with nucleotide analogues, qHBsAg levels showed a decline in parallel with HBV-DNA levels during treatment. There was a significant positive correlation between these two parameters. Our results suggest that qHBsAg levels can be used for treatment decision and monitoring. The reason why there was greater reduction in HBV-DNA levels may be the slower decline in HBsAg titers due to ongoing cccDNA presence, even though the viral replication is decreased. HBsAg titers were further reduced at the end of one year, which suggests that duration of treatment with nucleotide analogues should be longer.

Studies that investigate the significance of HBsAg level for monitoring treatment of chronic hepatitis B have mostly used interferon treatment. It has been reported that combination of qHBsAg and HBV-DNA levels may help to predict the early response to treatment with PEG-IFN in HBeAg-positive patients [18, 19]. Other studies also had similar results. In one multi-centered study in 2013 by Sonneveld et al. [20], they stated that qHBsAg levels could reliably be used for guidance on treatment decisions in HBeAg-positive patients treated with PEG-IFN. They also showed that the treatment could be discontinued in all patients if qHBsAg level at 24th week of treatment is greater than 20,000 IU/ml.

Since response rate to PEG-IFN treatment is lower in HBeAg-negative patients, prediction of persistent virological response is of greater significance; however, studies on this subject are limited. In light of the results of previous studies, it is recommended that treatment is discontinued if there is no decline in HBsAg and the decline in HBV-DNA level is less than 2 log at the 12th week of treatment in genotype D HBeAg (-) patients [21]. Marcellin et al. [22] found close association between end-treatment

response and decline in HBsAg levels at 12th and 24th weeks of treatment during a 5-year follow-up of HBeAg-negative patients receiving PEG-IFN alpha 2a treatment. Rijckborst et al. [23] evaluated treatment response with serum quantitative HBsAg levels in their study involving 107 HBeAg-negative patients receiving interferon treatment. They measured serum quantitative HBsAg levels at 4th, 8th, 12th, 24h, 36h, and 48th weeks of treatment and at the 72nd week following treatment. During the first 8 weeks of treatment, they had similar results both in treatment-responsive and treatment-non-responsive groups. Although HBV-DNA declined after the eighth week in both patient groups, the group that showed response to treatment had profound reduction in serum quantitative HBsAg levels, whereas the group that did not show response to treatment had still had high HBsAg levels.

In our study, 11 patients received PEG-IFN treatment. Mean initial HBV-DNA level in this group was 200.000.000±400.000.000 IU/ml. When compared to the initial levels, the decline in HBV-DNA was statistically significant both at 6th month ($p$=0.005) and at 12th month ($p$=0.01). The mean initial qHBsAg level was 56.160.77±88.683.82 IU/ml, qHBsAg level at the 6th month was 41.376.26±58.713.84 IU/ml, and qHBsAg level at 12th month was 24.509.11±49.331.21 IU/ml. When compared to the initial levels, it was statistically significant at the end of one-year follow-up ($p$=0.01). Although, the decline in qHBsAg level was not significant at the first six months ($p$=0.65). When we evaluated the correlation between serum qHBsAg and HBV-DNA levels in patients with chronic hepatitis B infection receiving PEG-IFN treatment, there was no significant correlation at the time of initiation and 6th month of treatment. However, there was a positive correlation between the two at the end of treatment. Of the 71 patients receiving treatment, 15 (21.1%) were HBeAg-positive and 56 (78.9%) were HBeAg-negative.

In our study, when we evaluated qHBsAg levels among HBeAg-negative and HBeAg-positive patients, we found a significant reduction in qHBsAg levels among HBeAg-positive patients (Table 2).

**Table 2.** The qHBsAg levels at the time of initiation, 6$^{th}$, and 12th month of treatment among HBeAg-positive and HBeAg-negative patients

| | HBe Ag-negative | HBe Ag-positive | *p* |
|---|---|---|---|
| Initial qHBsAg | 3.243.5 (1.250-6.903) | 65.390 (22.930-212.400) | 0.000 |
| 6th month qHBsAg | 2.837 (786.6-6.086.5) | 53.090 (24.310-153.200) | 0.000 |
| 12th month qHBsAg | 2.467 (600-5.251.5) | 21.230 (12.110-39.790) | 0.000 |

As a limitation to our study, we could not monitor results of long-term follow-up, since the study included only a one-year period. Additionally, it was aimed to observe the interaction with treatment, and not all patients could provide samples at the 6th and 12th months, which caused a limitation to the number of patients within the treatment groups.

When we evaluate our results together with the results of previous related studies, although monitoring of HBV-DNA levels is the most important parameter for assessment of treatment response in patients with chronic hepatitis B infection, it is not sufficient to predict the course of illness on its own. Considering that qHBsAg levels can better reflect cccDNA, it could be said that qHBsAg is a valuable parameter for treatment decision and for prediction of end-treatment response both in patients receiving nucleotide analogues and patients receiving interferon treatment. Our conclusion in this study is that qHBsAg levels can be used for monitoring treatment response.

**Conflict of interest:** No conflict of interest was declared by the authors.

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**Contributions of authors**

M.D. and N.T constructed the main idea and hypothesis of the study. M.D., N.T. and F.T. they developed the theory and organized the material method section. M.D., N.T., F.T., D.Y., S.E., G.TE made the evaluation of the data in the results section. Discussion section of the article written by M.D., F.T. and N.T. has reviewed and made the necessary corrections and approved. In addition, all authors discussed the entire study and confirmed its final version.