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HBV surface antigen loss prediction: A challenging yet achievable goal

Nada El-domiaty[1], Mohamed Adel El-Basiony[2,3], Ramy Hassan Agwa[2,4], Khaled Ghoniem[3], Nader Elmalky[2], Sherif Shiha[3], Reham Soliman[3,5*], Abdelkader Awadin[3]

[1]*Endemic Medicine Faculty of Medicine, Helwan Univ., Cairo, Egypt.*
[2]*Hepatology and Gastroenterology Unit, Internal Medicine dept., Faculty of Medicine, Mansoura Univ., El-Mansoura, Egypt*
[3]*Egyptian Liver Research Institute and Hospital (ELRIAH), Sherbin, El-Mansoura, Egypt*
[4]*Internal Medicine dept., Albaha Univ., Albaha, KSA*
[5]*Tropical Medicine dept., Faculty of Medicine, Port Said University, Port Said, Egypt*

Abstract

Introduction and aim: *Seroconversion of hepatitis B surface (HBs Ag) antigen is an infrequent event of cure during treatment of chronic hepatitis B virus (HBV) patients. It was estimated to be about 1-2% annually in Asian and Western cases. The aim of our study is to assess factors that could predict HBV surface (HBs) antigen loss in chronic HBV patients treated by nucleos(t)ide analogues.* ***Methods:*** *A total of 510 chronic HBV patients treated by (lamivudine, entecavir and tenofovir) with a median treatment time of 3.5 years (2.1-4.2 years). HBs antigen levels, HBV DNA levels, liver stiffness by FibroScan, liver functions tests, complete blood counts and prothrombin time were assessed at baseline and during follow up visits every 6 months.* ***Results:*** *Out of the 510 treated chronic HBV patients; 34 patients achieved HBs antigen loss. Multivariant regressive analysis showed that HBs antigen levels and HBV DNA levels were significantly lower in patients achieving HBs antigen loss compared to whom don't achieve with median [IQR] values of (2.53 [2.15-2.89] log_{10} IU/ml and 3.73 [3.66-3.83] log_{10} IU/ml) versus (3.24 [2.81-3.68] log_{10} IU/ml and 3.88 [3.69-4.22] log_{10} IU/ml), with p <0.001 and p= 0.022; respectively. Interestingly, 4/34 (11.8%) and 26/34 (76.5%) patients with HBs antigen loss had baseline HBs antigen levels below 100 IU/mal and ranging 100-1000 IU/ml respectively compared to 276/476 (58.0%) patients failed to achieve HBs antigen loss had baseline HBs antigen levels >1000 IU/ml (p value <0.001) and there were only 5 (14.7%) cases of cirrhosis (liver stiffness > 13.4 kPa) in HBs antigen loss group and 29 (85.3%) had no cirrhosis (p value 0.057). The cut-off points of basal HBs antigen quantitation to detect on-treatment HBs antigen loss was found to be 489 IU/ml with sensitivity 0.876 (95% CI 0.843 to 0.904), specificity 0.618 (95% CI 0.436 to 0.778), positive predictive value 0.970 (95 CI 0.939 to 0.977) and negative predictive value 0.262 (95% CI 0.213 to 0.436). The HBs antigen clearance group showed log_{10} reduction of HBs antigen quantitation by 0.42 after 24 weeks of treatment in comparison to 0.04 in the failure group (p value <0.001). Regarding treatment type in HBs antigen loss group; 19 cases was treated by entecavir and 15 by tenofovir and none of the Lamivudine treated patients achieved HBs antigen loss.* ***Conclusions:*** *Low HBs antigen, HBV DNA levels, and absence of liver cirrhosis could be considered as baseline predictors of HBs antigen loss. Rapid decline of HBs antigenemia with treatment and absence of treatment relapses are on-treatment indicators of HBs antigen clearance.*

Introduction

Chronic hepatitis B virus (HBV) infection remains a significant global health problem, affecting over 296 million people worldwide and leading to substantial morbidity and mortality due to liver cirrhosis and hepatocellular carcinoma (HCC)[1,2]. The antiviral treatment, with its different aims either HBe antigen seroconversion or HBV DNA loss, is favored with reduced rates of development of complications as HCC and liver cirrhosis when HBV DNA is effectively suppressed[3,4]. The HBV encodes three HBs antigen proteins; small, middle and large. These proteins form the viral envelope and are translated from two HBV sub-genomic mRNA transcripts, the preS1 mRNA and the preS2/S mRNA, in the endoplasmic reticulum (ER)[5,6]. The surface proteins also assemble to generate non-infectious excess sub-viral particles (SVP). The role of the SVPs in the pathogenesis of Chronic hepatitis B (CHB) is still unclear[7]. The covalently closed circular DNA (cccDNA), transcriptional template, drives reverse transcription of HBV[8]. This transcription generates all the mRNAs needed for HBV replication including SVP production. HBsAg may also be produced from HBV DNA integrated into the host genome[9]. The correlation between HBsAg titers and serum HBV DNA, and liver cccDNA was found, in most studies of HBe antigen positive patients, to be positive, but was lacking in HBe antigen negative patients[10-12]. Standardized HBsAg quantification in weight units per volume was firstly reported more than 40 years ago[13]. To predict disease activity and monitor treatment response in chronic HBV patients, there has been a lot of enthusiasm surrounding the use of serum HBs antigen quantification. The measurement of HBs antigen levels have been standardized in IU/ml. The development of new antiviral treatments aiming at HBs antigen (HBs Ag) seroclearance, meaning functional HBV cure, had led to mandating HBs antigenemia measurement[14]. A critical milestone in the management of chronic HBV infection is the loss of HBsAg, which is often considered a functional cure. However, achieving HBsAg loss remains a challenging goal due to the complex interplay of viral and host factors that influence HBV replication and immune response. Recent advancements in understanding the virology and immunology of HBV have provided new insights into potential predictors of HBsAg loss. These include baseline viral load, HBsAg levels, host genetic factors, and the efficacy of antiviral therapy. Despite these advancements,

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* **Corresponding author**. **email**: rehamelsayed@hotmail.com

the prediction of HBsAg loss is not straightforward and requires a multifaceted approach integrating clinical, virological, and immunological data[15]. This article explores the challenges associated with achieving HBsAg loss and discusses the current predictive markers that could aid clinicians in identifying patients who are more likely to achieve this milestone. By improving our ability to predict HBsAg loss, we can better tailor therapeutic strategies to enhance patient outcomes in chronic HBV infection.

Patients and Methods

Participants

This retrospective cohort study was done at Egyptian Liver Research Institute and Hospital (ELRIAH). A total of 510 consecutive chronic HBV patients during the period between January 2016 and December 2022 were recruited in the study. Patients with a history of concomitant human immunodeficiency, hepatitis delta or hepatitis C virus infections were excluded. The follow-up was conducted retrospectively and prospectively until December 2022. Data were collected from patients' files and electronic database after having the local institutional review board approval. Data comprised recipient's demographics including age, sex, smoking, diabetes mellitus and other chronic diseases collected initially together with alanine transferase (ALT), aspartate transferase (AST), liver stiffness and anti-HBV treatment. A 24-weekly assessment included basic clinical data, AST, ALT, CBC, abdominal ultrasonography, HBs antigen quantitation and HBV DNA testing by real time PCR.

HBV diagnosis

The presence of infection was screened by HBs antigen testing (qualitatively assessed by the AUSZYME monoclonal enzyme immunoassay [EIA] [Abbott Laboratories, Abbott Park, IL]). Infection was confirmed by real time PCR HBV DNA testing and revisited every 24 weeks. In patients with loss of HBsAg; seroconversion to anti-HBs was determined by the AUSAB EIA (Abbott Laboratories, Abbott Park, IL). Levels of HBsAg (IU/ml) in serum were quantified at the initial visit and every 24 weeks by the ARCHITECT assay (Abbott Laboratories, Abbott Park, IL), having a lower detection limit of 0.05 IU/ml (linear range 0.05–250 IU/ml).

Liver assessment

Liver stiffness was assessed using Fibroscan (Fibroscan Echosense, Paris, France) at the initial assessment. The procedure was performed by a single operator for all patients while fasting for at least 6 hours with 10 valid measures, success ate >70% and interquartile range/median value of < 0.03. Values of liver stiffness of > 8.7 KPa were used for significant fibrosis (≥ stage 2) and > 13.4 Kpa for liver cirrhosis (stage 4 fibrosis)[16].

HBV treatment

Nucleos(t)ide analogue treatment included Lamivudine, Entecavir and Tenofovir. Throughout the treatment period, all the patients received their anti-HBV treatment for free.

Study design (Classification of the patients according to the HBs antigen status)

Patients were classified according to the HBs antigen status into two groups; group 1: achieved HBs Ag loss group and group 2: non-achieved HBs Ag loss group. The patients of this study were randomized to the treatment type; Lamivudine, Entecavir and Tenofovir. Regular visits and follow up assessment through the follow up period were all collected including basic clinical data, AST, ALT, CBC, abdominal ultrasonography, HBs antigen quantitation and HBV DNA testing by real time PCR.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 26 (IBM Corp., USA). Continuous variables were reported as mean ± SD or median (IQR). Categorical variables were reported as frequency (%). Chi-square or Fisher's exact test were used for qualitative comparisons. Parametric analyses were performed by t-test or ANOVA while non-parametric analyses were performed with the Mann–Whitney test or Kruskal Wallis for quantitative data. Receiver operating characteristic (ROC) curve analysis with the calculation of the highest Youden index (sensitivity + specificity− 1) was used to calculate the best cutoff values for HBsAg for HBsAg clearance detection. P-values equal or less than 0.05 were considered statistically significant.

Results

A total of 510 treated chronic HBV patients were included. Based on HBs Antigen status, 34 patients (6.67%) achieved the functional cure of HBs Ag loss with a median treatment time of 3.5 years (2.1-4.2 years). There was no statistically significant difference between the different treatment groups regarding sex. Most of those patients (30/34, 88.2%) were HBe Ag negative and 4 patients (11.8%) were HBe Ag positive. 476 patients (93.3%) didn't achieve HBs Ag loss. Patients' demographic data, clinical characteristics and anti-viral treatment are summarized in **tab. 1.** Multivariant regressive analysis showed that HBs antigen quantification was found to be significantly lower in group 1 compared to group 2 (2.53 Vs 3.24 log IU/ml, p <0.001) Regarding HBs antigenemia in the 34 cases achieving HBs antigen loss; a range of 100-1000 IU/ml was found in 26 (76.5%), < 100 IU/ml in 4 (11.8%) and > 1000 IU/ml in 4 (11.8%). In the other hand, HBs antigenemia in the 476 cases not achieving HBs antigen loss, values of and > 1000 IU/ml in 276 (58.0%), 100-1000 IU/ml was found in 197 (41.4%) and < 100 IU/ml in 3 (0.6%) (p value < 0.001). HBV DNA levels were found to be significantly lower (p value 0.022) in cases achieving HBs antigen loss group (3.73 [3.66-3.83] Log10 – median [IQR]) in comparison to the failure group whom didn't achieve this target (3.88 [3.69-4.22] Log_{10} – median [IQR]). HBV DNA levels ranged 2000-20000 IU/ml in all the 34 cases achieving HBs antigen loss but in cases not achieving HBs antigen loss was 2000-20000 IU/ml in 370 (77.7%) cases, > 20000 IU/ml in 103 (21.6%) cases and < 2000 IU/ml in 3 (0.6%) cases (p value 0.008). Liver cirrhosis (using liver stiffness values > 13.4 Kpa by Fibroscan) was absent in 29 (85.3%) of the cases achieving HBs antigen loss but present in only 5 (14.7%) cases, but cases not achieving HBs antigen loss showed liver fibrosis in 143 (30.0%) cases (p value 0.057). Differences regarding treatment type were highly significant (p value <0.001), out of the 34 patients with HBs antigen loss; 0 (0.00%) were on Lamivudine, 19 (55.9%) on Entecavir and 15 (44.1%) on Tenofovir, **tab. 1.** **Table 2** shows the dynamic changes in virological response during the follow up period. HBV DNA levels were reduced to null after 24 weeks of treatment without any relapses during the follow up period in patients achieving HBs antigen loss while patients without HBs antigen loss remained positive during the first and 2nd follow up visits (p value = 0.031 and 0.643 respectively) and showed relapse in the fifth visit in 16

cases. In the other hand; the initial HBV DNA levels (before treatment) were significantly lower (P vale < 0.001) in patients achieving HBs antigen loss in comparison to patients not achieving this target (3.72 ± 0.14 and 4.13 ± 0.75 respectively), **tab. 2** & **fig. 1**. The dynamics of HBs antigenemia showed different pattern, patients whom had achieved HBs antigen loss showed maintained reduction in HBs antigenemia till loss with significantly (p value <0.001) with low initial level in comparison to patients without HBs antigen loss (2.5 [2.2-2.9] and 3.2 [2.8-3.7], mean [IQR] respectively). In contrast, the reduction of HBs antigenemia in patients not achieving HBs antigen loss showed a plateau in the reduction with higher initial levels, **tab. 2** & **fig. 2**. **Table 3** showed the biological and virological outcomes during the follow up period comparing the basic and 96-week results. There is highly significant reduction (p value <0.001) in ALT and AST levels (31.0 [23.0-46.0] to 27.0 [22.0-33.0] and 32.0 [23.8-46.3] to 29.2 [23.0-38.0], median [IQR], respectively). HBV DNA (log10) levels showed highly significant (p value <0.001) reduction to negative (32.0 [23.8-46.3] to 0.0 [0.0-0.0], median [IQR]). The reduction in HBs antigen quantitation was also highly significant (p value <0.001) (3.12 [2.78-3.59] to 2.90 [2.57-3.25], median [IQR]). Liver stiffness (by Fibroscan) showed highly significant reduction (p value <0.001) after 96 weeks of treatment (8.70 [5.40-13.83] to 5.15 [4.10-8.40], median [IQR]), **tab. 3**. Patients with HBs antigen loss showed high log_{10} reduction (-0.42 [-0.82 - -0.10]) of HBs antigenemia after 24 weeks of treatment in comparison to log_{10} reduction of -0.04 (-0.41 – 0.22) in patients failed to clear HBs antigen (p value < 0.001). In the other hand; the log_{10} reduction in HBV DNA levels showed no significant difference between the two groups (p value 0.169), **tab. 4**. Receiver operating characteristic (ROC) was used to determine the cutoff point of HBs antigen quantitation before treatment which can detect future on therapy HBs antigen clearance. The optimal cutoff point was 489 IU/ml with area under curve (AUC) 0.839. For a cutoff point of HBs antigen quantitation of 489 IU/ml; the sensitivity was 0.876 (95% CI 0.843 to 0.904), specificity 0.618 (95% CI 0.436 to 0.778), positive predictive value 0.970 (95 CI 0.939 to 0.977) and negative predictive value 0.262 (95% CI 0.213 to 0.436) for predicting on therapy HBs antigen clearance, **fig. 3** &. **tab. 5**.

Table 1 Patients characteristics at baseline and potential predictors for HBsAg loss

	Group 1 Achieved HBsAg loss (n=34)	Group 1 non-achieved HBsAg loss (n=476)	Un-variate P value	Multivariate p value
Female sex	11 (32.4%)	104 (21.8%)	0.157	
History of Diabetes Mellitus	8 (23.5%)	126 (26.5%)	0.707	
History of smoking	15 (44.1%)	241 (50.6%)	0.463	
ALT (U/L)	22.5 (19.0-40.0)	31 (24.0-47.0)	0.011	0.741
AST (U/L)	24.0 (16.0-34.5)	33.0 (24.0-48.8)	<0.001	0.057
Median Liver stiffness (kPa)	8.9 (5.7-10.4)	8.6 (5.4-14.2)	0.522	
HBe antigen ▪ ***Positive*** ▪ ***Negative***	 4 (11.8%) 30 (88.2)	 34 (7.1%) 442 (92.9%)	0.321	
Log_{10} HBV DNA	3.73 (3.66-3.83)	3.88 (3.69-4.22)	<0.001	0.022
HBV DNA (IU/mL) # ▪ ***<2000*** ▪ ***2000 – 20000*** ▪ ***>20000***	 0 (0.0%) 34 (100.0%) 0 (0.0%)	 3 (0.6%) 370 (77.7%) 103 (21.6%)	0.008	
Log_{10} (HBs Ag, IU/ml)	2.53 (2.15-2.89)	3.24 (2.81-3.68)	<0.001	<0.001
HBs Ag (IU/mL) # ▪ ***<100*** ▪ ***100-1000*** ▪ ***>1000***	 4 (11.8%) 26 (76.5%) 4 (11.8%)	 3 (0.6%) 197 (41.4%) 276 (58.0%)	<0.001	
Liver cirrhosis	5 (14.7%)	143 (30.0%)	0.057	
NUC treatment: ▪ ***Entecavir*** ▪ ***Tenofovir***	 19 (55.9%) 15 (44.1%)	 269 (56.5 %) 207 (43.5 %)	.915	

No: *number,* ***IQR:*** *interquartile range,* ***ALT:*** *alanine transferase,* ***AST:*** *aspartate transferase,* ***#:*** *Not included in multiple regression, and log of the actual value was included instead, NUC, nucleos(t)ide analogue* Categorical variables are expressed as number (%) and continuous variables as mean ± SD or median (IQR)

Table 2. Dynamic changes in virological response before during follow up period

	Group 1 Achieved HBsAg loss (n=34)	Group 1 non-achieved HBsAg loss (n=476)	P-Value
HBV DNA quantification			
Baseline	3.72 ± 0.14	4.13 ± 0.75	0.001
2nd	0.0 ± 0.0	0.42 ± 1.14	0.031
3rd	0.0 ± 0.0	0.02 ± 0.23	0.643
4th	0.0 ± 0.0	0.0 ± 0.0	NA
Last visit	0.0 ± 0.0	0.11 ± 0.59 #	0.278

HBs antigen quantification			
Baseline	2.5 (2.2-2.9)	3.2 (2.8-3.7)	<0.001
2nd	3.0 (2.7-3.3)	3.3 (3.0-3.6)	<0.001
3rd	2.9 (2.7-3.0)	3.4 (3.0-3.6)	<0.001
4th	2.5 (2.3-2.6)	3.0 (2.7-3.3)	<0.001
Last visit	0.0 (0.0-0.0)	2.9 (2.6-3.3)	<0.001

***#:** 16 patients out of 476 showed relapse in the 5th visit*

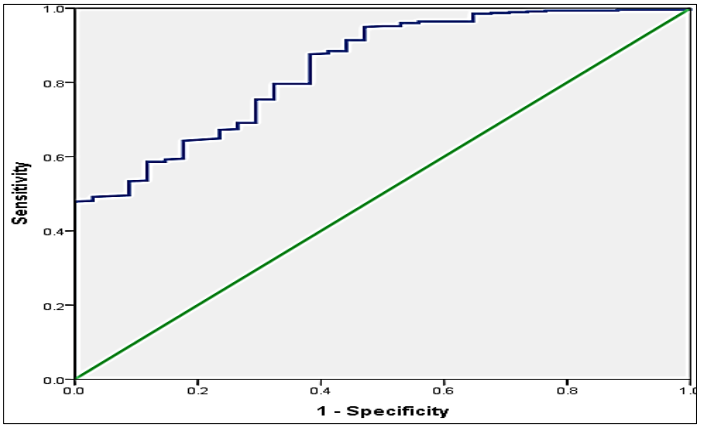

Figure 1. Dynamic changes of HBV DNA levels during the follow up visits (every 24 weeks)

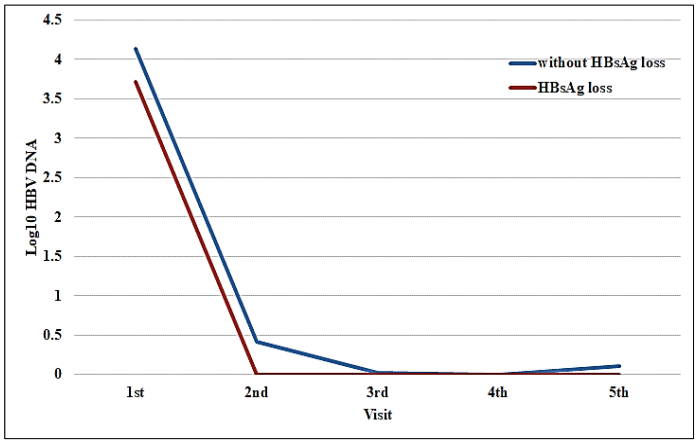

Figure 2. Dynamic changes of HBs antigen quantitation (Log_{10}) during the follow up visits (every 24 weeks).

Table 3. Outcomes

	Baseline	**Last visit (at 96 weeks)**	**p-value**
Biochemical response			
▪ ***ALT (U/L)***	31.0 (23.0-46.0)	27.0 (22.0-33.0)	**<0.001**
▪ ***AST (U/L)***	32.0 (23.8-46.3)	29.2 (23.0-38.0)	**<0.001**
Virological response			
▪ ***Log10 HBV DNA***	3.85 (3.69-4.21)	0.0 (0.0-0.0)	**<0.001**
▪ ***Log10 (HBs Ag, IU/ml)***	3.12 (2.78-3.59)	2.90 (2.57-3.25)	**<0.001**
Liver elasticity			
▪ ***Liver stiffness (Kpa)***	8.70 (5.40-13.83)	5.15 (4.10-8.40)	**<0.001**

***ALT:** alanine transferase, **AST:** aspartate transferase, **IQR:** interquartile range, **Kpa:** kilo pascal,* continuous variables are expressed as median (IQR).

Table 4. Log_{10} reduction of HBV DNA and HBs Ag up to week 24 post- NUC treatment

	Group 1 Achieved HBsAg loss (n=34)	**Group 2 non-achieved HBsAg loss (n=476)**	**p-value**
HBV DNA (log, IU/ml)	3.73 (3.66-3.83)	3.78 (3.57-4.09)	0.169
HBsAg (log, IU/ml)	-0.42 (-0.82 - -0.10)	-0.04 (-0.41 – 0.22)	**<0.001**

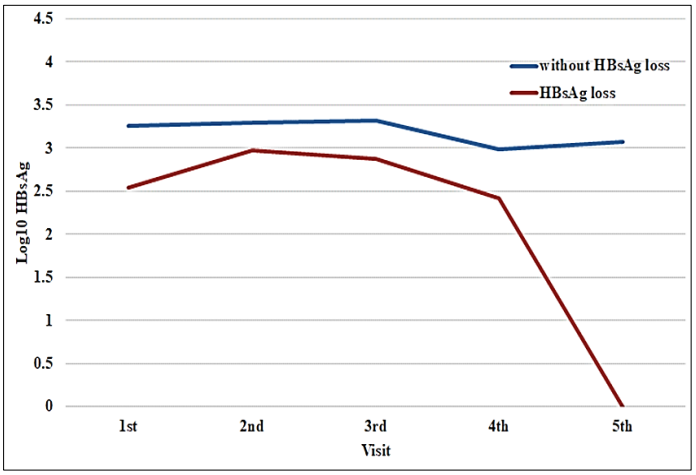

Figure 3. ROC curve for detection of HBS antigen quantitation cutoff point for detection of on therapy HBs antigen clearance. AUC of 0.839

Table 5. HBs Ag cutoff value for HBs Ag clearance detection after AUC treatment

Performance measures	Value	Lower limit	Upper limit
Sensitivity	0.876	0.843	0.904
Specificity	0.618	0.436	0.778
PPV	0.970	0.939	0.977
NPV	0.262	0.213	0.436
Optimal cutoff method: Youden **Optimal cutoff point: 489 IU/ml**			

PPV: *positive predictive value, NPV: negative predictive value*

Discussion

The hallmark of HBV infection is HBs antigen. It has been first discovered in 1968 and then used for diagnosis of HBV infection[17,18]. The template for both HBs antigen and HBV DNA synthesis is the cccDNA. HVB DNA quantitation merely reflects the activity of viral replication. In the other hand; HBs antigen quantitation in a standardized (IU/ml) manner can reflect and assess the cccDNA to supply a complementary data about HBV infection activity[19,20]. Regarding HBs antigen levels; higher antigenemia was found with HBe antigen positive, active disease and progression to complications like liver cirrhosis and HCC[20-23]. The current study reported 510 patients with chronic HBV infection with a median treatment period of 3.5 years (2.1-4.2 years). Only 36 (7%) of the patients were HBe antigen positive with the majority of cases were HBe antigen negative (474 cases). Only 34 (6.67%) cases achieved the important goal of HBs antigen loss, 30 of them were HBe antigen negative (88.2%) and only 4 were HBe antigen positive (11.8%). This goal is, Unfortunately, infrequent in cases with chronic HBV infection even with prolonged oral antiviral therapy[24-26].

HBs antigenemia

In the current study; low initial HBs antigenemia (Log_{10} IU/ml) was a strong predictor of achieving HBs antigen loss (p value < 0.001). Out of the 34 cases with HBs antigen loss; 30 (88.2%) had baseline HBs antigen levels below 1000 IU and only 4 had higher levels. In comparison; 276 (58.0%) out of 476 cases failed to achieve HBs antigen loss had HBs antigen levels > 1000 IU/ml (p value 0.001). High level of HBs antigenemia indicate more aggressive course of the disease with higher likelihood of developing complications as HCC and cirrhosis[19]. A HBs antigenemia < 100 IU/ml has a high specificity for cases with inactive chronic HBV infections and levels of < 1000 IU/ml may suggest inactive disease[23]. In a study by Wu et al; low baseline HBs antigenemia < 25000 IU/ml was found to be significantly associated with HBs antigen loss. This study was a retrospective study that was conducted on non-cirrhotics HBe antigen positive patients[30]. In their review; Mak et al. suggested that low HBs antigenemia may predict on-treatment or spontaneous HBs antigen seroclearance[27,28]. The cutoff value of HBs antigenemia to predict future HBs antigen loss was found to be 489 IU/ml with high sensitivity of 0.876 (95% CI 0.843 to 0.904) and high positive predictive value of 0.970 (95 CI 0.939 to 0.977) indicating that patients with HBs antigen quantitation around or less than this value are more promising of achieving HBs antigen loss, but the negative predictive value was low of 0.262 (95% CI 0.213 to 0.436) suggesting that patients with higher HBs antigenemia can achieve HBs antigen clearance. This is related to other aspects of the disease such as stage of the diseases, HBV DNA levels, liver fibrosis, viral resistance and genotype, response to treatment, treatment potency, and compliance to treatment.

HBV DNA levels

The current study showed that HBV DNA levels (Log_{10} IU/ml) were significantly lower in cases achieving the target of HBs antigen loss (p value 0.022) and all the 34 cases with HBs antigen loss had HBV DNA levels < 20000 IU/ml. Lower HBV DNA levels indicate less active disease with reduced replications. A previous study showed that patients with the combination of HBV DNA < 2000 IU/ml and HBs antigenemia < 100 IU/ml indicate inactive disease[23]. Also, the major predictor of HBs antigen loss over time was found to be low HBV DNA levels. This study tested spontaneous HBs antigen seroclearance and found that baseline HBV-DNA levels < 300 copies/mL had a cumulative probability of 69.3% in achieving this goal[29]. This was also shown in the study by Kim and

colleagues who found that high HBV DNA levels were highly inversely associated with HBs antigen seroclearance in patients treated with lamivudine and entecavir (P <0.001)[30]. Lower HBV DNA levels were found to be correlated with HBs antigen seroclearance[31]. In another study by Suzuki et al., 4 patients out of 27 cases achieving HBs antigen seroclearance had high baseline HBV DNA and ALT levels. These cases had acute infection at the time of treatment initiation[32]. Patients with lower viral load had better chance of viral clearance and HBs antigen loss and less risk of hepatitis relapse. HBs antigen levels were suggested to be better predictors of HBs antigen loss than HBV DNA levels [19].

HBs antigenemia Vs HBV DNA levels, better predictor of HBs antigen loss

In our study; HBs antigenemia was found to be highly significantly related to HBs antigen seroclearance in comparison to HBV DNA levels (p values <0.001 and 0.022 respectively). Seto et al., found that baseline HBs antigen level < 1000 IU/ml was the best indicator of subsequent HBs antigen seroclearance during lamivudine therapy[33]. It was found that the optimal level to predict spontaneous HBs antigen seroclearance is <10 IU/ml[19]. Lower levels of HBs antigenemia may be an indicator to immune controls with levels <1000 IU/ml pointing to moderate immune control that is augmented by NA therapy and suggesting that NA therapy may be required in patients with lower levels of HBs antigenemia to augment the immune response.

Liver cirrhosis

The current study showed that most patients with advanced liver fibrosis (using Fibroscan measures > 13.4) didn't clear HBs antigen during the follow up period and only 5 (14.7%) of the patients achieving HBs antigen loss had Fibroscan measures >13.4 Kpa indicating that advanced liver fibrosis/ cirrhosis is an indicator of failure of achieving this target. Although HBs antigen seroclearance was found to be associated with liver cirrhosis[34], EASL recommended that patients with compensated or decompensated cirrhosis need treatment, with any detectable HBV DNA level and regardless of ALT levels with a goal of regressing fibrosis and cirrhosis[35]. In patients with liver cirrhosis, the guidelines recommend to use entecavir or tenofovir because of their higher barrier for resistance[35, 36]. An analysis of cohorts of patients with compensated cirrhosis caused by HBV indicates that the disease decompensates at an annual rate of 1.5% to 5%. Following decompensation, the 5-year survival rate has varied from 14% to 35%[37]. Stopping nucleos(t)ide analogue treatment before HBs antigen loss in HBe antigen negative patients carries risks of flares and hepatic decompensation[38].

Dynamics of HBs antigenemia

The role of HBs antigenemia dynamics in patients receiving nucleos(t)ide analogues is still building up. The current study showed that both groups, with or without HBs antigen loss, showed highly significant (p value < 0.001) reductions in HBs antigenemia on treatment. Although this reduction was plateauing in the group with failure of HBs antigen loss and the initial visits showed some increase in the main HBs antigen quantifications, but the main difference was that the initial HBs antigen quantification was higher in the failure group (p value < 0.001). In our study; a HBs antigenemia $\log_{10}$ reduction of -0.42 (-0.82 - -0.10) was noted in patients with HBs antigen clearance in comparison to $\log_{10}$ reduction of -0.04 (-0.41 - -0.22) in cases failed to clear HBs antigen. Another study reported that HBe antigen negative patients under nuleos(t)ide analogue treatment showing a reduction of HBs antigen quantitation by > 1 $\log_{10}$ IU/ml were predicted to have HB antigen loss[14]. Similar results were found in another study where >log 10 reduction of HBs antigenemia was associated with HBs antigen seroclearance in patients using entecavir[32]. Regarding PEG-interferon therapy; it was shown that 1 log10 IU/ml reduction of HBs antigenemia at week 24 could be a predictor SVR (NPV ¼ 97%, PPV ¼ 92%). Also, sustained HBs antigen clearance was associated with low HBs antigen levels and 1 $\log_{10}$ IU/ml reduction of HBs antigenemia at week 48[39,40]. In contrast, failure of HBs antigenemia reduction at week 12 suggest that reaching HBV DNA levels <2000 or HBs antigen loss at week 24 is unlikely[14]. A previous study showed that patients with pronounced reduction in HBs antigenemia of > 1 $\log_{10}$ during the first 24 weeks of therapy are more likely to show HBs antigen loss[41]. This study was conducted on 266 HBe antigen patients treated with tenofovir disoproxil fumarate. In a study by Wursthorn et al. found that after 24 weeks of therapy, high rate of HBe antigen seroconversion was found in cases with rapid reduction of HBs antigen levels. In this study; 32 out of 162 patients showed > 1 $\log_{10}$ reduction of HBs antigenemia during the first year of telbivudine therapy, but only 8 of these 32 cases achieved HBs antigen loss at the 3rd year of therapy[42]. Wursthorn et al. study was conducted on HBe antigen positive cases only and only oral telbivudine was the treatment used. In another study by Seto et al. in HBe antigen negative chronic HBV patients; baseline and subsequent serial HBsAg levels had no association with virological relapse[43]. In Seto et al. Study; patients were using entecavir treatment an only 184 patients were recruited in a trial of treatment cessation. This study had few limitations. HBe antigen positive patients are few due to the low prevalence in Egypt. HBs antigen loss cases are minority. Genotyping was not performed in this study; however, HBV genotype D was reported to be the most prevalent among Egyptian HBV patients[44].

Conclusion

Low HBs antigen levels, HBV DNA levels, and absence of liver cirrhosis could be considered as a baseline predictor of HBs antigen loss. Rapid decline of HBs antigenemia with treatment and absence of on-treatment HBV DNA relapses are on-treatment indicators of possibility of HBs antigen clearance.

List of abbreviations

ALT: Alanine transferase
AST: Aspartate transferase
CHB: Chronic hepatitis B
cccDNA: Covalently closed circular DNA
ER: Endoplasmic reticulum
ELRIAH: Egyptian Liver Research Institute And Hospital
EIA: Enzyme immunoassay
HBV: Hepatitis B virus
HBs Ag: Hepatitis B surface Antigen
HCC: Hepatocellular carcinoma
SVP: Sub-viral particles

Authors contributions

Conception and design by M. El-Basiony and R. Soliman; *Clinical work and procedures performed* by R. Agwa, S. Shiha and K. Ghoniem; *Statistical analysis conducted* by M. El-Basion and N. Elmalky; *Data collection* by M. El-Basiony, R. Soliman, and S. Shiha and A. Awadin; *Data analysis and interpretation* by M. El-Basiony and N. Elmalky; *Manuscript*

writing by M. El-Basiony, N. El-Domiaty, R. Soliman, and S. Shiha; *Revision and final approva*l by all authors.

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