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Original article

Hepatitis B Surface Antigen Quantitation as a Predictor of Treatment Response in Chronic Hepatitis B

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Abstract:
Hepatitis B surface antigen loss (HBsAg) is a primary therapeutic aim in the management of chronic hepatitis B (CHB), HBsAg levels may be a surrogate marker of infected cells in the liver, and is considered as the only possible quantitative assay for intrahepatic viral load in treated patients with undetected viremia. A rapid HBsAg decline during nucleoside/nucleotide (NA) therapy may identify patients who will show clearance of HBsAg. Currently, there is no consensus on the clinical utility of serum HBsAg monitoring for evaluating patient responses to NA therapy. We aimed to evaluate the possible value of HBsAg quantitation for prediction of treatment response to oral antiviral therapy. This prospective study was carried out on 130 CHB patients treated with oral antiviral therapy in outpatient clinic of Internal Medicine Department, Mansoura University Hospital and Egyptian Liver Research Institute and Hospital during the period from March 2011 to March 2015. Eighty five patients were on Lamivudine, 27 patients were on Entecavir, 11 patients were on Tenofovir and 7 patients were on Adifovir. Patients were checked every 6 months after starting treatment for 3 years. Twelve out of 130 patients partially responded to the therapy, 9 cases were break through, 103 cases responded and 6 cases achieved HBsAg loss, the baseline HBsAg level has a high predictive value for HBsAg loss vs other patients (AUROC= 0.870, P < 0.01) at cutoff value ≤1927.50 IU/mL with a sensitivity of 83.3%, a specificity of 73.4%, a PPV of 83.15%, and an NPV of 98.9%. While cut off value of ≤128.50 after 6 months of treatment has a sensitivity of 83.3%, a specificity of 98.4%, a PPV of 71.4%, and an NPV of 99.2%. Significant HBsAg decline to 128.5 IU/ml after starting treatment is useful predictor for patients that can achieve HBsAg loss.

1. Introduction

Chronic hepatitis B (CHB) is a major global health problem and a leading cause of liver-related complications, including cirrhosis, liver failure, hepato-cellular carcinoma (HCC), and death. The goal of nucleoside/nucleotide (NA) analog therapy for CHB is to suppress the replication of hepatitis B virus (HBV)

in a sustained manner, and prevent disease progression to decompensated cirrhosis and HCC [1]. The most frequent markers for treatment monitoring are HBeAg seroconversion, biochemical response (no-rmalization of serum ALT) and virological response (HBV DNA <2000 IU/ml to undetectable).However, all these markers have limitations. HBeAg can only be used in HBeAg-positive CHB, while HBeAg-negative CHB is increasingly frequ-ent. Although HBV DNA quantification is the most widely used marker to predict treatment response, prediction of a sustained off treatment response, using an on-treatment decrease in viral load during PEG-IFN therapy is difficult since the patterns of decrease in HBV DNA are similar in sustained responders and relapsers. Hepatitis B Virus DNA becomes rapidly undetec-table in patients who receive NAs while only 50% of these patients will become HBeAg-negative and most will relapse once treatment is stopped [2]. The hepatitis B surface antigen (HBsAg) was identified more than 50 years ago by Blumberg et al. in 1965 [3]. Detection of HBsAg in serum is still the hallmark of HBV infection and the cornerstone for the diagnosis of CHB. Over the years, HBsAg quantific-ation has been shown to be are liable marker to predict clinical outcome [4]. HBsAg quantification was considered to be a promising, simple and inexpensive method to monitor viral replication in CHB patients treated with IFN. Studies have reported that the management of CHB can be optimized in daily practice using HBsAg quantification [4-6]. Indeed, availability of standardized commercial assays has renewed interest in quantitative serum HBsAg as a biomarker to stratify the risk of disease progression and predict treatment response, mainly inpatients receiving PEG-IFN therapy. However, HBsAg quantification cannot replace viral load measurement in clinical practice. The combination of both titers has been shown to be useful for monitoring the natural history and outcome of the disease [7]. The aim of this work was to study the possible value of HBsAg quantitation as a predictor of treatment response to NA in chronic hepatitis B patients.

2. Patients and Methods

2.1. *Study design*

The present prospective study was carried out on 130 patients with chronic HBV infection attending the outpatient clinic of Internal Medicine Department, Mansoura University Hospital and Egyptian liver Hospital during the period from March 2011 to march 2015, after Ethical approval for the study by the Scientific Council of Internal Medicine Department, Egypt Board for Medical Specification and obtaining adequate consent.

2.2. *Inclusion criteria*

Patients aged 18-60 years with chronic HBV infection, HbeAg negative, HBV DNA level more than 2000 IU or histological activity more than A1, fibrosis more than F1 in liver biopsy using metavir scoring system. We consider also patients with family history of HCC and partners with HBV if one of them is for treatment we treat both, even with low viral load.

2.3. *Exclusion criteria*

A known allergy to one of the used drugs, decompensated liver cirrhosis (ascites, varices, encephalopathy, albumin level < 3 mg/dL, total bilirubin level > 2.5 mg/dL, or prothrombin time > 3 seconds longer than normal), another cause of clinically significant liver disease, or the presence of hepatocellular carcinoma, kidney, heart, or lung transplant, autoim-mune hepatitis and coinfection with hepatitis C and delta virus.

2.4. *Methods*

A signed informed consent was obtained from all subjects eligible for the study at screening time after ethical committee approval. All subjects gave a thorough medical history, and received a complete clinical examination. The overall eligibility of the subjects to participate in the study was assessed. Investigations in the form of:

1- Laboratory investigations including;

*****Complete blood picture.

*****Liver enzymes (ALT and AST).

*****Liver function tests (total Bilirubin, total Protein and serum albumin).

*****HBV DNA levels were quantified with a real-time PCR assay on a Cobas TaqMan 48 analyzer (Roche Molecular Systems, Branchburg, NJ); the lower detection limit was 16 IU/mL.

*****HBsAg quantitation ARCHITECT QT assay (Abott Diagnostic, Wiesbaden, Germa-ny) according to the manufacturer's instructions, the assay was carried out in two steps: HBsAg present in the sample was bound to antibody to hepatitis B surface antigen (anti-HBs)–coated micro particles, and an acridinium-labeled anti-HBs conjugate was added together with pretrigger and trigger solutions. The products of the resulting chemilumin-escent reaction were measured in relative light units. The qHBsAg calibration curve ranged from 0.05 to 250 IU/mL, and the samples were diluted with a diluent (1:20 or 1:250) as needed to expand the detection range. HBeAg was also determined by Architect platform (Abbott Diagnostic). *****HCV Ab, Delta Ig, ANA.

2- Abdominal ultrasound.

3- Fibroscan using cut off value 8.4 Kpa for less than F2, 9,6Kpafor less than F3 and 12.8 Kpa for F4 [8].

4- Liver biopsy performed for patients with PCR above 2000 IU with normal enzymes and patients below 2000 IU with elevated enzymes.

2.5. *Methodology of liver biopsy*

Under complete aseptic condition patient on left lateral position using 16G true cut needle, local anesthesia (Lidocain 3-5 ml) was injected in the intercostal space at the site determined by abdominal us for the right lobe of the liver away from any vessels or ducts, the biopsy taken saved in formalin and send to the pathologist in the same day.

2.6. *Follow-up visits*

After baseline data and samples collection (visit 1) Patients were checked every 6 months after starting treatment (Visit 2.3.4.5) by physical examination, HBsAg level, HBV DNA, ALT, AST, CBC and abdominal ultrasound.

2.7. *Treatments*

Drugs used are:

1- Lamivudine 100mg tablets, once daily.

2- Adifovor 10mg tablets once daily.

3- Entecavir 0.5mg tablets once daily or 1mg for relapsers.

4- Tenfovir 300 mg tablets once daily.

2.8. *Drug Resistance*

Primary non-response or virological breakthrough was managed according to EASL 2012 guidelines for HBV treatment. Resistant to lamivudine switch to tenfovir or add on adifovir or switch to Entecavir 1gm. Resistant to adfovir switch to enticavir 1gm.

2.9. *Responses definitions*

The primary endpoint of this study was the serial analysis of qHBsAg profiles in patients receiving treatment. VR was defined as an HBV DNA level undetectable by a real-time PCR assay

(<16 IUs/mL) at 24 months. Virological breakthrough was defined as an increase in HBVDNA levels to ≥1 log IUs/mL from the treatment nadir after a decline of≥2 log IUs/mL. Primary non-response was defined as an HBV DNA decline of <2 log IUs/ mL after 6 months of therapy. While HBsAg loss is considered as cure in our study.

2.10. *Statistical Analysis*

The statistical analysis of data was done using excel program(Microsoft Office 2013) and SPSS (statistical package for social science)program (SPSS, Inc, Chicago, IL) version 20.Qualitative data were presented as frequency and percentage. Chi square and Fisher's exact tests were used to compare groups. Quantitative data were presented by mean, SD or median and range. Comparisons between two groups were done using t-test or Man Whitney (for nonparametric), while compareison between more than two groups were done using ANOVA or Kruskal Wallis tests (for non-parametric). To determine the best cutoff for maximal accuracy, we applied receiver operating characteristic (ROC) curves and areas under the curve (AUROCs). The sensitivity and specificity of HBs Quantity & HBV-DNA to differentiate between different groups were examined at different cutoff points using ROC curve analysis. The correlation between variables was analyzed with r. P value less than 0.05 was considered to be statistically significant.

3. Results

One hundred and thirty patients were enrolled, and their serial samples were analyzed. The baseline characteristics of the patients is shown in tab. (1). After baseline data and samples collection (visit 1) Patients were checked every 6 months after starting treatment (Visit 2,3,4,5) by physical examination, HBsAg level, HBV DNA, ALT, AST, CBC and abdominal ultrasound. ALT normalization was observed in visits 4 and 5, while AST levels were normal after first visit. All patients had elevated baseline ALT and AST levels regarding the WHO normal values of liver enzymes ALT have been defined as below 30 U/L for men and 19 U/L for women.<2 log IUs/ mL after 6 months of therapy. Partial response defined as decline of HBV DNA level ≥2 log IUs/ml after 6 months of treatment while DNA level still detected (<16 IUs/mL), tab. (2). The Median HBV DNA decline was marked after first visit of therapy. Median HBV DNA levels were measured as follows: 3935, 0.0,0.0, 0.0 and 0.0 IU/ml, for visits 1-5 respectively. The corresponding median HBsAg quantitation results were 7356.50, 5113.50, 4430.00,5167.00 and 3090.00 IU/ml, for visits 1-5 respectively, tab. (3). Virological response was defined as an HBV DNA level undetectable by a real-time PCR assay (<16 IUs/mL) at 24 months. Virological breakthrough was defined as an increase in HBV DNA levels to ≥1 log IUs/mL from the treatment nadir after a decline of ≥2 log IUs/mL. Primary non-response was defined as an HBV DNA decline of<2 log IUs/ mL after 6 months of therapy. Partial response defined as decline of HBV DNA level ≥2 log IUs/ml after 6 months of treatment while DNA level still detected (<16 IUs/mL). Twelve out of 130 patients partially responded to the therapy, 9 cases were break through, 103 cases responded and 6 cases were have achieved (HBsAg loss) i.e. cure tab. (4). The median HBV DNA decline is marked during the early phase of therapy in patients who responds and those with complete cure compared to other groups. HBV DNA levels were elevated in patients with partial response to therapy in the first visits then decline slowly in following visits. In patients with breakthrough, an increase in HBV DNA levels to152 IUs/mL at visit 3 and 3441.5 IUs/mL at

visit 4 from the treatment nadir after a decline from 6090 IUs/mL in first visit to 0.0 IUs/mL in visit 2. Responders and cured patients show seroclearance of HBV DNA after first visit. HBsAg decline was stronger during the early phase of therapy inpatients with complete cure compared to other groups. HBsAg levels were elevated in patients with partial response to therapy on first visits then declined slowly in following visits. In break through an increase in HBsAg levels to 11127.0 IU/mL at visit 3 and 9288.0 IU/mL at visit 4,11767.0 IU/mL at visit 5 from the treatment nadir after a decline from 7634.0 IU/mL in first visit to 5779.0 IU/mL in visit 2. Cured patients show complete disappearance of HBsAg after the third visit (tabs. 5 and 6 and fig. 1). Table (7) shows that, HBsAg percent change from previous visit was significantly high in patient with complete cure between first and second visit and second and third visit with decline in HBsAg level (69.36% and 64.20%) respectively. In breakthrough group there is rise in the HBsAg level between the second and third visits and between third and fourth visits (57.24% and 7.33%) respectively. Partial responders show fluctuating percent of change, while responders shows relatively low decline than cure (17.5%) between first and second visits. Our results have shown that the baseline HBsAg level has a high predictive value for complete response vs other patients (AUROC= 0.870, P< 0.01) and cutoff value ≤1927.50 IU/mL with a sensitivity of 83.3%, a specificity of 73.4%, a PPV of 83.15%, and an NPV of 98.9%. When compared to HBV DNA (AUROC= 0.68, P < 0.05) and cutoff value≤1885.50 IU/mL with a sensitivity of 66.7%, a specificity of 73.4%, a PPV of 70.8%, and an NPV of 97.9%. While cut off value of ≤128.50 in second visit in patients with complete cure has with a sensitivity of83.3%, a specificity of 98.4%, a PPV of 71.4%, and an NPV of 99.2%, tab. (8).

Table (1) Baseline characteristics of studied cases

Parameter	***No.***	***%***
Age		
mean ± SD	36.08 ± 8.60	
- ***<40 y***	81	62.3
- ***> 40 y***	49	37.7
Sex		
- ***Male***	105	70.8
- ***Female***	25	19.2
Family history		
- ***None***	115	88.5
- ***Father***	1	0.8
- ***Mother***	0	0.0
- ***Sister***	0	0.0
- ***Brother***	12	9.2
- ***Son***	2	1.5
Treatment		
- ***Lamivudine***	85	65.4
- ***Entecavir***	27	20.8
- ***Tenofovir***	11	8.5
- ***Adifovir***	7	5.4

Table (2) Laboratory characteristics of studied cases in different visits

Parameter	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 4*	*Visit 5*
Hb	13.54±1.47	13.71±1.35	14.03±1.27	14.29±1.13	14.65±.60
WBC x10^3	6.86±2.33	7.09±1.43	6.86±1.51	6.96±1.57	7.12±1.20
Platelets x10^3	206.92±57.52	220.45±60.64	237.38±62.04	225.91±52.61	215.25±12.5
ALT	33.00±6.25	30.00± 4.65	32.00± 4.55	23.00± 2.33	23.00±2.32
AST	32.00± 5.24	28.00±3.45	29.00± 3.66	27.50±4.22	24.00±3.19
Albumin	4.26±0.50	4.37±0.48	4.28±0.34	5.16±1.20	4.7±0.65
Bilirubin	1.11±1.00	0.94±0.46	1.07±1.93	0.80±0.38	0.82±0.14
INR	1.07±0.11	1.06±0.09	1.20±1.90	1.04±0.09	1.02±0.03
S. Creatinine	0.70±0.60	0.70±0.4	0.70±0.52	0.70±0.58	0.70±0.62

Data are expressed as mean±SD

Table (3) HBV DNA and HBsAg quantitation among studied patients in different visits

Parameter	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 4*	*Visit 5*
HBV-DNA					
- Median	3935.00	0.00	0.00	0.00	0.00
- Range	1720.00-88800.0	0.00-772.00	.00-59.30	0.00-0.00	0.00-134.00
HBs Quantity					
- Median	7356.50	5113.50	4430.00	5167.00	3090.00
- Range	1331.0-15407.0	1133.0-10980.0	1209.0-9864.0	1037.0 -13080.0	866.0-4189.0

Table (4) Response to therapy

Response Status	*No.*	*%*
Responder	103	79.2
Partial response	12	9.2
Break through	9	6.9
HBsAg loss (Cure)	6	4.6

Table (5) Comparisons between types of responder in HBV-DNA in different visits

HBV-RNA	**Response**				**P**
	Responder	***Partial response***	***Break through***	***Cure (HBsAg loss)***	
Visit 1					
- Median	3760.00	500320.00	6090.00	1511.00	0.5
- IQR	2000.0-59300.0	640.0-1000000.0	1720.0-249000.0	1040.0-6040.0	
Visit 2					
- Median	0.00 [a]	323820.0	0.00 [a]	0.00 [a]	<0.001
- IQR	0.00-264.00	3640.0-644000.0	0.00-0.00	0.00-.00	
Visit 3					
- Median	0.00 [ab]	1597.50	152.00 [a]	0.00 [ab]	<0.001

- IQR	0.00-.00	990.0-2205.0	0.00-342.00	0.00-.00	
Visit 4					
- Median	0.00 [ab]	353.00	3441.50	0.00 [ab]	<0.001
- IQR	0.00-.00	353.0-353.0	0.00-25610.00	0.00-.00	
Visit 5					
- Median	0.00 [ab]	134.00	624.50	0.00 [ab]	<0.001
- IQR	0.00-.00	134.00-134.00	251.00-998.00	0.00-.00	

Data are expressed as median, IQR (interquartile range) - P: Probability Significance when<0.05 - Test used: Kruskal Wallis followed by Mann-Whitney for pairwise comparisons - a: significance relative to Partial response - b: significance relative to Breakthrough - c: significance relative to responder

Table (6) Comparisons between types of responder in HBs-Quant in different visits

HBs-Quantitation	**Response**				**P**
	Responder	***Partial response***	***Break through***	***Cure (HBsAg loss)***	
Visit 1					
- Median	5004.00 [a]	24464.0	7634.00 [a]	587.00 [abc]	<0.001
- IQR	1164.0-15881.0	9928.0-39000.0	2130.0-9011.0	100.0-1841.0	
Visit 2					
- Median	3804.00 [a]	22461.00	5779.00 [a]	85.0 [abc]	<0.001
- IQR	996.0-9734.0	10980.0-33942.0	4800.0-576.0	28.00-88.00	
Visit 3					
- Median	4423.00 [a]	19495.50	11127.0	16.00 [abc]	<0.001
- IQR	1015.0-10477.8	8777.0-30214.0	6763.0-11366.0	0.00-63.0	
Visit 4					
- Median	4636.00 [a]	20974.00	9288.00	0.00 [abc]	<0.001
- IQR	1033.5-11310.0	9078.0-32870.0	8268.0-16845.0	0.00-200.0	
Visit 5					
- Median	3170.00 [a]	17960.00	11767.0	0.00 [a]	<0.001
- IQR	595.0-11788.0	7896.0-28024.0	1373.0-22161.0	0.00-0.00	

Data are expressed as median, IQR (interquartile range) - P: Probability Significance - zhen<0.05 - Test used: Kruskal Wallis followed by Mann-Whitney for pairwise comparisons - a: significance relative to Partial response - b: significance relative to Breakthrough - c: significance relative to responder

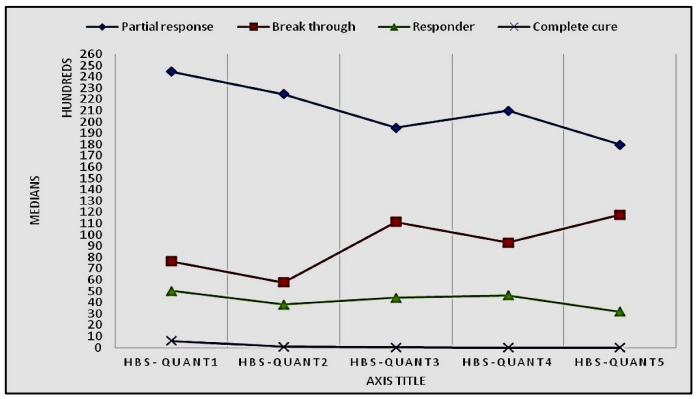

Figure (1) HBsAg level change in different type of response to treatment

Table (7) Comparisons between types of responder in percent changes of HBsAgQuantitation in different visits

Percent changes	*Response*			
	Responder	***Partial response***	***Break through***	***Cure (HBsAg loss)***
2nd change	--	--	--	--
- Median	-17.50	-1.19	-24.30	-69.36[ac]
- IQR	-53.75-40.48	-12.97-10.60	-46.73-124.09	-85.52- -12.00
3rd change	--	--	--	--
- Median	-1.68- [b]	-15.52-	57.24 [a]	-64.20- [abc]
- IQR	-35.25-57.68	20.06- -10.98	32.53-90.44	-100.00- -25.88
4th change	--	--	--	--
- Median	-8.79-	6.11	7.33	-100.00-
- IQR	-25.57-31.80	3.43-8.79	-52.43-48.21	-100.00-525.00
5th change	--	--	--	--
- Median	-8.52-	-13.88	69.66	-100.00-
- IQR	-26.60-4.83	-14.74- 13.02	-28.71-168.03	-100.00--100.00

Data are expressed as median, IQR (interquartile range)
P: Probability Significance when<0.05
Test used: Kruskal Wallis followed by Mann-Whitney for pairwise comparisons
a: significance relative to Partial response
b: significance relative to Breakthrough
c: significance relative to responder

Table (8) ROC curve, cutoff values, sensitivity, specificity, and accuracy of HBsAg quantitation versus HBV DNA to response of therapy

HBsAg loss vs other	AUROC (CI95%)	Cutoff value	Sensitivity	Specificity	PPV	NPV	Accuracy
- ***HBs Quant1***	0.87(0.76-0.97)	≤1927.50	83.3	73.4	83.15	98.9	73.8
- ***HBs Quant2***	0.95(0.87-1.00)	≤128.50	83.3	98.4	71.4	99.2	97.7
- ***HBV DNA***	0.68(0.49-0.86)	≤1885.5	66.7	73.4	70.8	97.9	73.07
Responder vs others	0.65(0.53-0.76)	≤9889.00	64.1	61.9	89.18	26	63.7
Partial vs others	0.8(0.71-0.89)	≥9889.0	100	67.8	24.0	100.0	70.8
Break through vs others	0.54(0.42-0.67)	≤7717.0	66.7	48.8	8.8	95.16	50.0

AUROC=Area under the curve
HBs Quant 1= (visit 1). HBs Quant2= (visit 2)

4. Discussion

This prospective study was carried out on 130 patients with chronic HBV infection attending the outpatient clinic of Internal Medicine Department, Mansoura University Hospital and Egyptian Liver Research Institute and Hospital during the period from March 2011 to march 2015. In the current study, the median HBV DNA decline was stronger during the early phase of therapy in patients who responded and those with cure compared to other groups. HBV DNA levels were high inpatients with

partial response to therapy on first visits then decline slowly in following visits. In patients with breakthrough, an increase in HBVDNA levels to 152 IU/mL at visit 3 and 3441.5 IU/mL at visit 4 from the treatment nadir after a decline from 6090 IU/mL in first visit to 0.0IU/mL in visit 2. Responders and cured patients show seroclearance of HBV DNA at second visit. These results were in concordance of Gheorghita et al. [9] who found stronger HBV DNA decline during the early phase of therapy in patients who developed sustained virological response compared to those without SVR. The profile of HBV DNA decline was similar inpatients who achieved SVR and in patients who developed relapse following an initial end of treatment response. They found the mean value of viral load at 12 weeks of treatment was significantly lower in patients who developed SVR in comparison with relapsers. Our results revealed that the median HBsAg decline was stronger during the early phase of therapy in patients with cure compared to other groups. HBsAg levels were high in patients with partial response to therapy on first visits then decline slowly in following visits. In patients with breakthrough, an increase in HBsAg levels to 11127.0 IU/mL at visit 3 and 9288.0 IU/mL at visit 4 and 11767.0 IU/mL at visit 5 from the treatment nadir after a decline from 7634.0 IU/mL in first visit to 5779.0IU/mL in the second visit. Cured patients show complete disappearance of HBsAg after third visit. These results were closer to Gheorghita et al. [9] findings who found that HBsAg level decreased significantly through the 48 weeks course of therapy (the mean decline was 0.48 log10IU/ml, p=0.003). However, patients who obtained complete cure experienced a significantly more important decrease in serum HBsAg titer. They found, the mean declines of serum HBsAg in this subset of patients at 12, 24 and 48 weeks were 0.5 ± 1 log10 IU/ml, 0.6 ± 1 log10IU/ml and 1 ± 1.3 log10 IU/ml, respectively. On the contrary, the nonresponder patients did not achieve a signifycant decrease in serum HBsAg level during treatment (the mean decline at week 48 was 0.04 ± 0.5 log10IU/ml). In fact, the mean level of HBsAg was almost the same as the baseline value in this category of patients. In patients who developed end of treatment response, the profile of serum HBsAg kinetics was different in patients who achieved SVR compared to those who relapsed. Thus, the serum HBsAg level did not decline during treatment in relapsers, having the same profile as nonresponder patients. In this study, HBsAg percent change from previous visit was significantly high in cured patient between first and second visit and second and third visit with decline in HBsAg level (69.36% and 64.20%) respectively. In breakthrough group there is rise in the HBsAg level between the second and third visits and between third and fourth visits (57.24% and 7.33%) respectively. Partial responders show fluctuating percent of change, while responders show relatively low percent of decline than cure (17.5%). These results were in agreement with Burczynska et al. [10], they reported that responder patients had significant decline in HBsAg concentrations of baseline values compared to partial or breakthrough responders. Of interest in the current study is the pattern of HBsAg decline which is different in patients who achieved break through response at second visit, compared to those with HBsAg loss (cure). In break through the decrease in serum HBsAg during treatment was minor compared with the significant decrease of HBsAg levels recorded in patients who achieved HBsAg loss. Basically, breakthrough had the same HBsAg declining profile as partial responder patients. These observ-ations reinforce the idea that HBsAg can be considered as an alternative biomarker for immunological control of HBV infection and response to

treatment. Our results have shown that the baseline HBsAg level has a high predictive value for HBsAg loss (cure) vs other patients (AUROC= 0.870, P< 0.01) at cutoff value ≤1927.50 IU/mL with a sensitivity of 83.3%, a specificity of 73.4%, a PPV of 83.15%, and an NPV of 98.9% with accuracy of 73.8, (AUROC= 0.950, P < 0.01) and cutoff value ≤128.50IU/mL with a sensitivity of 83.3%, a specificity of 98.4%, a PPV of 71.4%, and an NPV of 98.9 % with accuracy of 97.7 in second visit. When compared to HBV DNA (AUROC= 0.68, P < 0.05) and cutoff value≤1885.50 IU /mL with a sensitivity of 66.7%, a specificity of 73.4%, a PPV of 70.8%, and an NPV of 97.9% with accuracy of 73.07. These results are similar to Lee et al. [11], they found that the baseline cutoff level HBsAg of 9550 IU has AUROC = 0.823 (P < 0.001) with a sensitivity of 86.8%, a specificity of 78.9%, a PPV of 89.2%, and an NPV of 75.0%. Additionally, Weng et al. [12] also found that the cutoff of 6000IU/mL of serum HBsAg at months 6 had a PPV of 73.3% and an NPV of96.8% for predicting combined responses of ALT normalization, HBVDNA negativity and HBeAg seroconversion. Our current study showed that the best indicator of response to HBV therapy was the baseline HBsAg level (AUROC 0.870) with an optimal cutoff value <1927.5 IU/mL, and at second visit HBsAg level (AUROC0.950) with an optimal cutoff value <128.5 IU/mL strengthening the clinical applicability of serum HBsAg measurements during HBV therapy. Low HBsAg levels specifically after treatment reach <128.5IU/mL could reflect a perfect response of treatment and even to the extent of viral clearance. This level is similar to the optimal level used to predict spontaneous HBsAg seroclearance in CHB, which is identified within the range of 10 to 200 IU/mL [13,14]. Seto et al. [15] evaluated the reduction of HBsAg Levels and HBsAg seroclearance in chronic hepatitis B patients receiving 10 years of nucleoside analogue therapy; they found that baseline serum HBsAg achieved an AUROC of 0.860, better than the AUROC for the rate of HBsAg reduction (0.794). The optimal baseline HBsAg level and rate of HBsAg reduction to predict NA-related HBsAg seroclearance were 1,000 IU/ mL(sensitivity, 85.7%; specificity, 84.1%; PPV, 37.5%; NPV, 98.1%) and0.166 log IU/mL/year (sensitivity, 83.3%; specificity, 73.0%; PPV,26.1%; NPV, 97.8%), respectively. In addition, serum HBsAg <128.5 IU/mL in second visit in predicting HBsAg seroclearance achieved a high NPV (99.2%), and hence performing HBsAg measurements at the commencement of HBV therapy could identify patients with low probability of subsequent HBsAg seroclearance even when successful virologic suppression is achieved. As for patients with a high baseline HBsAg (>1927.5 IU/mL) or failing to achieve a significant HBsAg decline to 128.5 IU/ml 6-12months after treatment, HBsAg seroclearance during our therapy would be an improbable treatment response. Nonetheless, novel treatment options (e.g., HBsAg release inhibitors) are currently undergoing clinical trials, thus treatment related HBsAg seroclearance could still be a reachable target for such patients in the future.

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