Evaluation of serum hepatitis B surface antigen quantification kinetics in different patterns of chronic hepatitis B infection

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Background and aim
Management of chronic hepatitis B (CHB) infection is challenging owing to its wide immunologic phases and pathologic patterns. Quantification of serum HBs-Ag (qHBsAg) has been used as a mirror of intrahepatic viral replication, and therefore, its kinetics in these patterns may reflect diseases severity and hence may help in tailoring the management plan. So, this study was designed to assess the patterns and kinetics of qHBsAg among Egyptian patients presented with different forms of CHB.

Patients and methods
Between December 2016 and December 2017, patients with CHB were enrolled and categorized into three groups. Group I included naive aviremic patients [those with negative hepatitis B virus (HBV)-DNA-PCR without treatment]. Group II included naive low viremic patients (those with HBV-DNA-PCR <2000 IU/ml without treatment). Group III included treatment-experienced aviremic patients (those with negative HBV-DNA-PCR after 6 months of nucleotide analog treatment). All patients were checked for alanine aminotransferase (ALT), qHBsAg, and HBV-DNA-PCR at a regular intervals: at baseline (W0), week 12 (W12), and week 24 (w24).

Results
A total of 90 patients were enrolled, with 30 patients in each group. In group I, qHBsAg and ALT levels exhibited relatively stable detectable positively correlated levels from W0 to W24. In group II, qHBsAg and ALT levels were the same as group I. In group III, qHBsAg, ALT levels, and HBV-DNA exhibited a significant decrease during the follow-up with the use of nucleotide analog therapy.

Conclusion
qHBsAg measurement, which is a cheap and easy test, can replace HBV-DNA and may help in reflection of disease activity and assessment of follow-up.

Keywords:
chronic hepatitis B infection, inactive carrier, quantification of serum hepatitis B surface antigen

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Introduction

Chronic hepatitis B (CHB) infection is considered one of the major public health problems [1], and its management is faced by a dilemma of when and how to assess disease activity and treatment responses [2]. Hepatitis B surface antigen (HBsAg) is a glycosylated envelope protein of hepatitis B virus (HBV) virion that is synthesized from translated messenger RNAs of transcriptionally active covalently closed circular DNA (cccDNA), which needs liver sampling to be measured [3]. Many trials were designed to assess qHBsAg as a mirror for intrahepatic replication of the virus and to measure cccDNA, and so, can replace liver biopsy [4,5].

Therefore, we aimed to measure the kinetics of qHBsAg in different forms of CHB infection among Egyptian patients.

Patients and methods

Patients' recruitment and characteristics

Between December 2016 and December 2017, a prospective study was designed to include all adult patients with well-proven CHB. Assessment was done in accordance to the guidelines.

All patients had document presence of hepatitis B surface antigen in serum for more than 6 months and had an age range of 20 and 54 years. Patients were selected from those attending the Viral Hepatitis Outpatients Clinic of Al-Rajhi University Hospital during the study period from December 2016 and

December 2017. Any patient with manifestation of liver cell failure or co-infected with other viral hepatitis was excluded.

Enrolled patients were subdivided into three groups (30 patients in each of them).

(1) Group I: naive aviremic patients (those with negative HBV-DNA-PCR without treatment)
(2) Group II: naive low viremic patients (those with HBV-DNA-PCR <2000 IU/ml without treatment)
(3) Group III: treatment-experienced aviremic patients (those with negative HBV-DNA-PCR after 6 months of nucleotide analog treatment for at least 6 months duration).

Study methods

For all patients, CHB workup (HBsAg, anti-HBcAb, HBeAg, and anti-HBeAb) was done by commercially available ELISA kits (Abbott Diagnostics, IL, USA)). Serum alanine aminotransferase (ALT) was tested to evaluate the hepatic necroinflammatory insult. Quantification of serum HBsAg was done by RocheCobas e 411 analyzer with Elecsys HBsAg (all methodology as per Elecsys package inserts for quantitative HBsAg measurement, Quant reagent kits'; Roche Diagnostics, Indianapolis, Indiana, USA). HBV-DNA-PCR was tested by real-time PCR using the QIAampMinElute Virus Spin kit (QIAGEN, Hilden, Germany).

For all patients, qHBsAg, ALT, and HBV-DNA-PCR were serially repeated at baseline (week 0; W0) and then at week 12 (W12) and at week 24 (W24).

Statistical analysis and ethical considerations

Data was collected and analyzed those using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data were expressed in the form of mean ± SD or median (range), whereas categorical data were expressed in frequencies (percentage). Pearson correlation test was used to determine the correlation between qHBsAg with HBV-DNA and ALT. *P* value was considered statistically significant if less than 0.05. This study was approved by our Local Ethical and Research Committee, Faculty of Medicine, Assiut University.

Results

A total of 90 patients were enrolled. Their mean age was 40.45 ± 10.23 years, with an age range of 20–54 years. The patients comprised 82% males.

Group I: naive-aviremic patients

The qHBsAg and ALT levels in those patients were significantly changed (by fluctuation in their levels) at third and sixth month of follow-up in comparison with the baseline level ($P < 0.05$), whereas HBV-DNA was negative (undetected) during the period of study (Table 1). Moreover, the level of qHBsAg had positive significant correlation with ALT at baseline and at sixth month of follow-up (Fig. 1).

Group II: naive low viremic patients

The levels of qHBsAg, HBV-DNA, and ALT were significantly changed (by fluctuation in their levels) at third and sixth of follow-up in comparison with the baseline level (Table 2). The qHBsAg level had a negative significant correlation with HBV-DNA level at baseline and at sixth month of follow-up, whereas it had a positive significant correlation with ALT at baseline and at sixth month of follow-up (Fig. 2).

Group III: treatment-experienced aviremic patients

It was noticed that quantitative HBsAg level in group III patients was significantly decreased at third and sixth of follow-up in comparison with the baseline level. Moreover, HBV-DNA level in patients was significantly decreased at third and sixth of follow-up in comparison with the baseline level ($P = 0.02$). The same improvement occurred with ALT (Table 3).

It was noticed that quantitative HBsAg level had insignificant correlation with HBV-DNA level at baseline and weak positive correlation at sixth month of therapy, but it had a positive significant correlation with ALT at baseline and at sixth month of therapy (Fig. 3).

Discussion

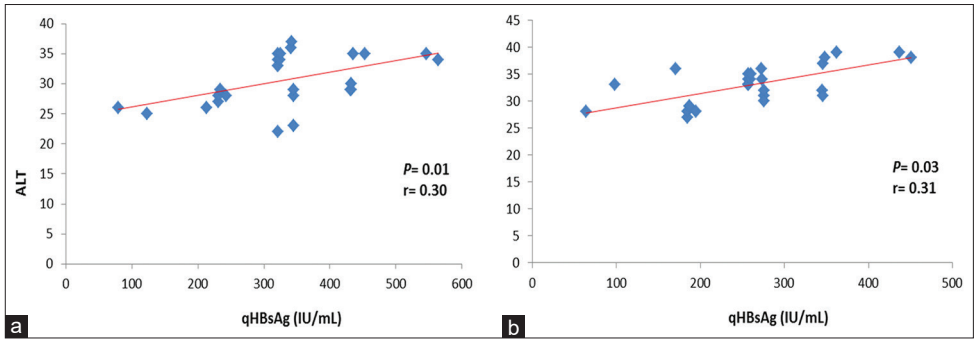
The longitudinal follow-up of ALT and HBV-DNA levels and/or assessment of liver histology in CHB patients is considered the main important factor in determining the phases of CHB infection and the guide for best treatment choice. However, this method is not applicable to the limited-resource countries because of the high cost involved and the need for frequent testing [6]. Recent studies have incorporated

Table 1 Laboratory data for group I patients

	qHBsAg (IU/ml)	HBV-DNA (IU/ml)	ALT (U/l)
W0	534.12±67.45	Undetected	29.11±6.89
W12	234.34±87.34	Undetected	22.04±9.03
W24	443.28±123.45	Undetected	33.22±10.34
P	0.01	-	0.02

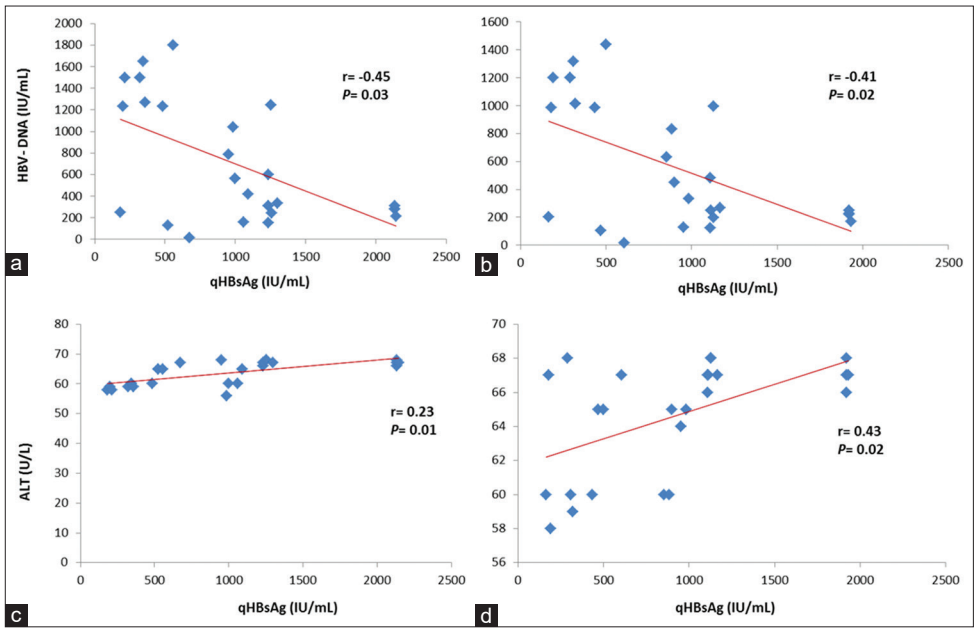
Data are expressed as mean±SD. *P* value is significant if <0.05. ALT, alanine aminotransferase; HBVDNA, hepatitis B virus DNA; qHBsAg, quantitative hepatitis B surface antigen.

Figure 1



Correlation of quantitative hepatitis B surface antigen with alanine aminotransferase at baseline (a) and at sixth month of follow-up (b) in group I.

Figure 2



Correlation of quantitative hepatitis B surface antigen with hepatitis B virus-DNA at baseline (a) and at sixth month of follow-up (b) and with alanine aminotransferase at baseline (c) and at sixth month of follow-up (d) in group II.

the use of qHBsAg to classify the patients and to predict antiviral therapy response and also have created a new role for serum qHBsAg in clinical practice [7]. Moreover, qHBsAg (qHBsAg) was introduced as a mirror that may reflect the integrated cccDNA inside the hepatocytes nucleus [8,9].

So, in this study, we aimed to evaluate the kinetics of qHBsAg among Egyptian patients with different forms of CHB. A well-defined grouping was designed to cover all possible patients with different spectrum of CHB, either treatment naïve or experienced. In patients with spontaneous negative viremia, qHBsAg and ALT levels were significantly changed (by fluctuation in their levels) at W12 or W24 of follow-up in comparison with the baseline level, with persistently negative HBV-DNA (undetected) all over the study period. To the best of our knowledge, we did not find any published article that tested qHBsAg among this unique group. So our result may be the first to tackle this issue. Interestingly, in spite of negative viremia,

Table 2 Laboratory data in group II

	qHBsAg (IU/ml)	HBV-DNA (IU/ml)	ALT (U/l)
W0	1000.31±231.32	700.21±213.65	65.01±13.98
W12	632.01±125.34	560.54±219.3	50.12±6.89
W24	900.28±234.32	1121.98±218.11	62.01±12.98
P	0.02	0.02	0.01

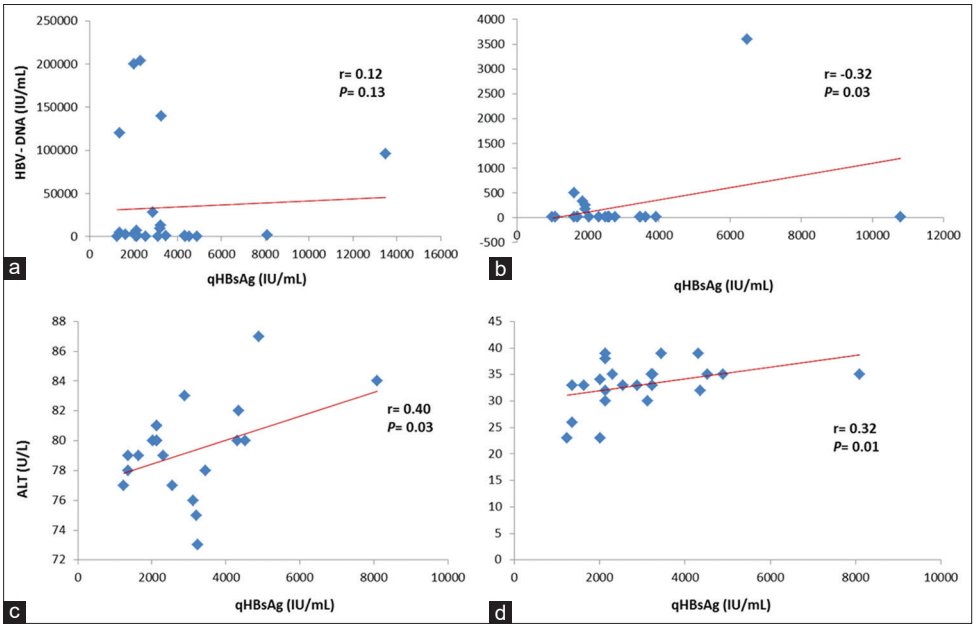
Data are expressed as mean±SD. *P* value is significant if <0.05. ALT, alanine aminotransferase; HBVDNA, hepatitis B virus DNA; qHBsAg, quantitative hepatitis B surface antigen.

Table 3 Laboratory data in group III

	qHBsAg (IU/ml)	HBV-DNA (IU/ml)	ALT (U/l)
W0	3411.50±897.32	34883.31±8454.43	78.42±12.56
W12	2729.87±548.09	4325.54±1243.45	55.11±9.46
W24	156.77±66.11	198.6±19.81	33.01±5.09
P	0	0.02	0.02

Data are expressed as mean±SD. *P* value is significant if <0.05. ALT, alanine aminotransferase; HBVDNA, hepatitis B virus DNA; qHBsAg, quantitative hepatitis B surface antigen.

Figure 3



Correlation of quantitative hepatitis B surface antigen with hepatitis B virus-DNA at baseline (a) and at sixth month of follow-up (b) and with alanine aminotransferase at baseline (c) and at sixth month of follow-up (d) in group III.

we found a fluctuation in the level of qHBsAg, which may denote the persistence of viral particle integrated inside the hepatocyte, and this may indicate the need for long-term follow-up of this group for the fear of viral flare. This finding meets with the conclusion of the study of Tanaka *et al.* [10] on a similar group of patients with CHB (HBsAg positive and HBV-DNA negative), as they concluded that undetectable level of DNA might not accurately reflect the true proportion of subviral particles vs virions.

In the second group, low viremic naive patients, we found the same fluctuation between qHBsAg and ALT during the follow-up in comparison with the baseline level. Moreover, qHBsAg level had a negative significant correlation with HBV-DNA level at baseline and at sixth month of follow-up, but it had a positive significant correlation with ALT level at baseline and at sixth month of follow-up. In the same context, Gunal *et al.* [11] reported that the values of qHBsAg levels were significantly higher in patient with positive HBV-DNA than those with undetectable HBV-DNA level, and also they found a weak significant correlation between qHBsAg and ALT level. Balkan *et al.* [12], who evaluate correlation between serum qHBsAg, ALT, and HBV-DNA levels, in the inactive carrier and CHB, reported different finding. Their study revealed no correlation was noted between serum qHBsAg, ALT, and HBV-DNA levels, for inactive HBV carriers and HBeAg-negative patients with CHB. However, a moderate positive correlation was determined between serum qHBsAg levels and HBV-DNA in the HBeAg-positive patients with CHB.

In patients who received oral NAs, levels of qHBsAg and HBV- DNA were significantly decreased during the follow-up. Moreover, qHBsAg level showed insignificant correlation with HBV-DNA level at baseline and a weak positive correlation at sixth month of therapy, which may reflect the potency of the treatment on the viral replication and cccDNA. This notion was also reported by Fung *et al.* [2] where the profound HBV-DNA suppression was not associated by significant change in qHBsAg of most patients. A possible explanation for this can be explained by the fact that in spite of the potency of oral nucleotide analog, they did not affect the cccDNA integration [1,2]. In agreement with our study, Gish *et al.* [13] reported that no correlation was observed between on-treatment changes in qHBsAg and changes at HBV-DNA. This observation was consistent with other studies assessing the relationship between HBV-DNA levels, qHBsAg levels, and serological responses in HBeAg-positive patient treated with oral NAs.

In our study, the lack of correlation between the on–treatment kinetics of qHBsAg and serum HBV-DNA can be possibly explained by the same fact of the lack of potency of NAs on cccDNA and its sole action on the inhibition of HBV-DNA replication [13,14].

Conclusion

In conclusion, the incorporation of qHBsAg assays was found to be a quite useful test when used together with ALT and HBV-DNA levels in distinguishing inactive

carrier from patients with CHB. Moreover, it can replace the repetition of expensive tests, especially in those who need long-term follow-up. More intense research studies are needed to elucidate the potential role of qHBsAg assay in tailoring treatment responses and strategies.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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