Correlation between HBsAg Quantitation Assay, Histopathology and Viral - DNA Level in Chronic HBV Patients

Adel A.Mostafa[1], Mohammed E.El-Shewi[1], Hatem S. Abd El-Raouf[1], Waleed A. Elagawy[2], Mohammed A.El-Rashidy[3],Hala G.Shouman[4]

[1] *Hepatology, Gastroenterology and Infectious Diseases Department, Faculty of Medicine, Benha University ,Egypt.*
[2] *National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt.*
[3] *Pathology Department, Faculty of Medicine, Tanta university, Egypt.*
[4] *Hepatology Department,Mahalla Teaching Hospital, Al-Mahalla Alkoubra, Egypt.*

Corresponding Author
Hala Gamal Shouman

Mobile:
+201272228912

E mail:
Shouman5555@gmail.com

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Background and study aim : Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world. In this regard, Egypt has an intermediate HBV seroprevalence. Quantitation of hepatitis B surface antigen (HBsAg) by automated chemiluminescent micro-particle immunoassay has been proposed to be a surrogate marker. The aim of this study was to evaluate correlation between HBsAg quantitation assay, histo-pathology and viral-DNA level in chronic hepatitis BeAg negative patients.

Patients and methods: This study carried on 50 HBV-DNA positive naïve CHB patients. All the studied patients were HBeAg negative. HBV DNA was measured by real-time polymerase chain reaction, and serum HBsAg was quantified by electrochemiluminescence assay (Roche Diagnostic). Liver biopsy done to all patients in this study for histopathological grading and staging of hepatic fibrosis by experienced pathologist using METAVER scoring.

Results: A significant correlation between serum HBsAg quantitation level and HBV DNA levels was revealed in all studied HBeAg negative patients (r =0.748, P= <0.001). A significant correlation between serum HBsAg level and fibrosis (r=0.334, P= 0.018). While, there is no significant correlation between serum HBsAg quantitation level and activity by Metaver scoring in all studied patients (P >0.05).

Conclusion: All the studied patients were tested for assess correlation between HBsAg quantitation, HBV-DNA level and histo-pathology grading and staging. There is strong positive correlation between HBsAg quantation level and HBV-DNA and between HBsAg quantation level and stages of fibrosis. No correlation between HBV DNA levels and histopathology.

INTRODUCTION

Approximately one third of the world's population has serological evidence of past or present infection with HBV and 350-400 million people are chronic HBV surface antigen (HBsAg) carrier **[1]**.

Clinical course of chronic hepatitis B (CHB) is highly variable, ranging from an asymptomatic carrier state, to the development of cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) **[2]**.

The prevalence of chronic hepatitis B virus infection is about 5% worldwide, but it differs between regions, 0.1%-02.0% in the United States and western Europe, 2.0%- 8.0% in Mediterranean countries and Japan, and 8.0%-20% in southeast Asia and Sub-Saharan regions **[3]**.

Egypt has an intermediate HBV sero-prevalence. HBV is classified into ten different genotypes (A-J) with different geographic distributions. Genotype D is the most prevalent in the Middle East. Limited data are available about HBV genotyping among Egyptian blood donors, particularly in Upper Egypt **[4]**.

Hepatitis B surface antigen (HBsAg) is considered a milestone in the research history of hepatitis B virus (HBV) and has been used for more than 40 years to confirm HBV infections **[5]**.

Recently, the relationship between serum HBsAg concentrations and HBV-DNA levels in hepatitis B patients who are positive for serum HBsAg and HBeAg was examined. Serum HBsAg concentration was related to HBV-DNA replication level **[6]**.

The architect HBsAg assay was the first to be approved and is widely used; it is a chemi-luminescence microparticle immunoassay (CMIA) in which serum and anti-HBs- coated paramagnetic microparticles are combined **[7]**.

PATIENTS AND METHODS

Study design :
Cross-sectional study**.**

Patients :
We enrolled in the study 50 naive patients with Chronic hepatitis B virus, consecutively observed at Hepatology department at Mahalla Teaching Hospital in the period between May 2013 to October 2014, fulfilling all criteria detailed below.

Inclusion criteria :
Patients were included if they had a diagnosis of HBV based on HBsAg, HBeAg, HBV-DNA level, Liver biopsy. Male or female age 18 years or older.

Exclusion criteria :

- Exclusion of any other case of liver disease other than HBV :
  - Co-infection with HCV.
  - Hepatic focal lesion or other malignancy.
- Decompensated liver disease.
- Alcoholic liver disease (history of alcohol intake).
- Obesity induced liver disease
- Pregnancy or breast feeding.

All patients were divided into two groups (A&B) :

- **Group A:** Including ;(25) patients with Chronic hepatitis B virus subdivided into two sub groups:
  - Subgroup **(A1)** : HBV-DNA <2000 IU/ml with normal ALT.
  - Subgroup **(A2)** : HBV- DNA<2000 IU/ml with persistently elevated ALT (at least for 3 months interval).
- **Group B:** Including (25) patients with Chronic hepatitis B virus Subdivided into two sub groups :
  - Subgroup **(B1)**: HBV-DNA ≥ 2000 IU/mL with normal ALT.
  - Subgroup **(B2)**: HBV-DNA ≥ 2000 IU/mL with persistently elevated ALT.

All patients were subjected to the following :

1- Full history taking and through clinical examination.
2- Laboratory investigations including :
   - Complete blood count (CBC), Liver function tests, HBeAg, HBsAg Quantitation by chemiluminescent microparticle assay and Alpha-feto-protein (IU/ml).
   - HBV DNA measurement by real time PCR (Roche): HBV-DNA levels were expressed in IU/ml.

1- Liver biopsy: will be done for histo-pathological grading and staging of hepatic fibrosis by experienced pathologist using METAVER scoring using special stain (Masson's trichrome stain). Examples of liver biopsy already done for chronic hepatitis B patients in this study reading by histo-pathologist using hematoxylin and eosin (H&E) staining and Masson's trichrome stains as shown in (Figs. 1,2).

Statistical analysis :
The collected data were tabulated and analyzed using SPSS version 16 software (SPSS Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Chi square test (X^2), Spearman's correlation coefficient (rho), ANOVA and Krauskal Wallis test were the used tests of significance. ROC curve was used to determine cutoff values with optimum sensitivity and specificity. The accepted level of significance in this work was stated at 0.05 ($P<0.05$ was considered significant).

RESULTS

Fifty treatment naive patients with chronic hepatitis B (CHB) (persistent HBsAg positive for 6 months), recruited into the current study and divided into two groups; group (A) included patients with HBV DNA <2000 IU/ml, while group (B) included patients with HBV DNA ≥2000 IU/ml.

Group (A) included 18 male and 7 female with their mean age (30.92 ± 5.64 years), while group (B) included 19 male and 6 female with their

mean age (29.24 ± 4.25years). No significant statistical difference was founded between both groups regarding age and gender as shown in (Table 1).

In group (A) the mean value ±SD of Hb was (14.12 ± 1.88), WBCs (5.65 ± 1.38) and for platelets was (198.72 ± 61.14), while in group (B) the mean value ±SD of Hb was (13.22+1.55), WBCs (5.89 ± 1.51) and platelets was (194.44 ± 59.69). No significant difference was found between both groups regarding the previously mentioned parameter, the mean value ±SD of total bilirubin in all groups was normal in group (A) (0.84 ± 0.30) and in group (B) (1.00 ± 0.34 mg/dl). The mean value ±SD of prothrombine time was normal in both groups. ALP values were not exceeding upper limit of normal in both groups, the mean value of ALP all groups was not significant. The mean value ±SD of AFP (alpha fetoprotein) in all groups was normal in group (A) (2.84 ± 2.18) and in group (B) (2.50 ± 1.41) as shown in (Table 2).

In both groups (A: HBV-DNA <2000 IU/ml, B: HBV-DNA ≥2000 IU/ml), there is strong correlation between HBsAg quantitation and HBV-DNA levels with mean value ±SD (155.28 ± 59.48) and (5359.52 ± 1754.90) respectively with strong statistical significant (p= 0.001) as shown in (Table 3 and Fig.3).

There is no correlation between HBV DNA level among all studied groups which (<2000 IU/ml or ≥2000 IU/ml) with histopathplogy grading and staging by METAVER scoring (p= 0.467) (Table 4).

In all studied patients with normal ALT (≤ 40 IU/Ml) (group (A1): 13 patients with HBV DNA level <2000 IU/ml & group (B1): 12 patients with HBV DNA level ≥2000 IU/ml), there is no statistical significant difference regarding histo-pathology by METAVER scoring (fibrosis, p= 0.198 & activity, p=0.490) as shown in (Table 5).

In all studied patients with persistently elevated ALT (>40 IU/ml) (group (A2): 12 patients with HBV DNA level <2000 IU/ml & group (B2): 13 patients with HBV DNA level ≥2000 IU/ml), there is no statistical significant difference with histopathology by METAVER scoring (fibrosis, p= 0.192 & activity, p=0.545) as shown in (Table 6).

The study of correlation between HBsAg quantitation and histopathology in all studied groups, showed there is statistical significance between fibrosis and HBsAg quantitation (r= 0.334, p=0.018) and no correlation with activity (r=0.017, p=0.905) by metaver scoring (fibrosis and activity) as shown in (Table 7).

In the studied group, a cut-off HBsAg titer of 1730.85 IU/ml could predict serum HBV DNA levels ≥ 2000 IU/ml with 100% sensitivity, 100% specificity, P value <0.001 and area under the curve (AUROC= 1.00), as shown in (Table 8 and Fig. 4).

Table (1) : Comparison between patients with HBV DNA level below 2000 IU/ml & above 2000 IU/ml regarding age and gender

Parameter	**Group (A) HBV DNA<2000 IU/ml (n = 25)**		**Group (B) HBV DNA≥2000 IU/ml (n = 25)**			**P**
	No	**%**	**No**	**%**		
Sex :						
Male	18	72%	19	76%	$\chi 2 = 0.104$	0.747
Female	7	28%	6	24%		
Age (in years)	30.92 ± 5.64		29.24 ± 4.25		t = 1.189	0.240
BMI (kg/m2)	30.92 ± 5.64		29.24 ± 4.25		t = 1.189	0.240

Table (2) : Comparison between patients with HBV DNA level below 2000 IU/ml & above 2000 IU/ml regarding complete blood count and liver profile.

Liver function tests	Group (A) HBV DNA<2000 IU/ml (n = 25)	Group (B) HBVDNA≥2000 IU/ml (n = 25)	t	P
Hb (gm/dl)	14.12 ± 1.88	13.22 ± 1.55	1.867	0.068
WBCS ($x10^3$ /mm^3)	5.65 ± 1.38	5.89 ± 1.51	0.586	0.560
Platelets ($x10^3$ /mm^3)	198.72 ± 61.14	194.44 ± 59.69	0.250	0.803
T. Bilirubin (mg/dl)	0.84 ± 0.30	1.01 ± 0.34	1.773	0.083
D. Bilirubin (mg/dl)	0.38 ± 0.23	0.49 ± 0.22	1.838	0.072
Albumin (gm/dl)	4.32 ± 0.51	4.11 ± 0.54	1.407	0.166
AST (IU/ml)	35.64 ± 17.18	40.24 ± 26.55	0.727	0.471
Prothrombin time (%)	92.83 ± 9.31	89.16 ± 10.61	1.300	0.200
Alk Ph (mg/dl)	155.16 ± 51.95	167.32 ± 58.03	0.781	0.439
AFP (> 20ng/ml)	2.84 ± 2.18	2.50 ± 1.41	0.656	0.516

Table (3) : Correlation between HBV DNA level &HBsAg quantitation

Parameter	Group A HBV DNA<2000 IU/ml (n = 25)	Group B HBV DNA≥2000 IU/ml (n = 25)	t	P
HBsAg Quantitation (IU/ml)	155.28 ± 59.48	5359.52 ± 1754.90	14.819	<0.001*

Table (4) : Comparison between patients with HBV DNA level below 2000 IU/ml and above 2000 IU/ml regarding Histopathology

Parameter	Group A HBV DNA<2000 IU/ml (n = 25)	Group B HBV DNA≥2000 IU/ml (n = 25)	t	P
Histopathology Metaver fibrosis				
(F0)	0 (0%)	0 (0 %)	χ^2 = 4.352	0.113
(F1)	19 (76%)	12 (48%)		
(F2)	4 (16%)	10 (40%)		
(F3)	2 (8%)	3 (12%)		
(F4)	0 (0%)	0 (0%)		
Histopathology Metaver activity				
(A0)	0 (0%)	0(0%)	χ2 = 1.524	0.467
(A1)	21 (84%)	20 (80%)		
(A2)	3 (12%)	5 (20%)		
(A3)	1 (4%)	0 (0%)		

Table (5) : Comparison between the studied groups regarding HBV DNA level & Histopathology with normal ALT (≤ 40 IU/ml)

Parameter	Group A HBV-DNA<2000Iu/ml (n = 13)	Group B HBV-DNA≥2000Iu/ml (n = 12)	χ2	P
Histopathology: Metaver fibrosis			3.239	0.198
(F0)	0 (0%)	0 (0%)		
(F1)	11(84.6%)	8(66.7%)		
(F2)	1 (7.7%)	4(33.3%)		
(F3)	1 (7.7%)	0 (0%)		
(F4)	0 (0 %/)	0 (0%/)		
Histopathology: Metaver Activity:			0.476	0.490
(A0)	0 (0%)	0 (0%)		
(A1)	12(92.3%)	10(83.3%)		
(A2)	1 (7.7%)	2 (16.%)		
(A3)	0 (0%)	0 (0%)		

Table (6) : Comparison between the studied groups regarding HBV DNA level & Histopathology with elevated ALT (> 40 IU/ml)

Parameter	Group A HBV-DNA<2000Iu/ml (n = 12)	Group B HBV-DNA≥2000Iu/ml (n = 13)	χ2	P
Histopathology: METAVER			3.299	0.192
(F0)	0 (0%)	0 (0%)		
(F1)	8 (66.7%)	4 (30.8%)		
(F2)	3 (25%)	6 (46.2%)		
(F3)	1 (8.3%)	3 (23.1%)		
(F4)	0 (0%)	0 (0%)		
Histopathology: Metaver activity			1.215	0.545
(A0)	0 (0%)	0 (0%)		
(A1)	9 (75%)	10 (76.9%)		
(A2)	2 (16.7%)	3 (23.1%)		
(A3)	1 (8.3%)	0 (0%)		

Table (7): Comparison between Hepatitis B surface quantitation antigen and Histopathology in all studied groups

Variables	Hepatitis B surface antigen quantitation	
	r	p
Activity	0.017	0.905
Fibrosis	0.334	0.018*

Table (8) : ROC curve for Hepatitis B surface antigen for detection of level of Hepatitis B DNA-PCR ≥2000IU/ml

Variable	Results
Area under the curve	1.00
P value	0.001
Cut off point	1730.85
Sensitivity	100%
Specificity	100%

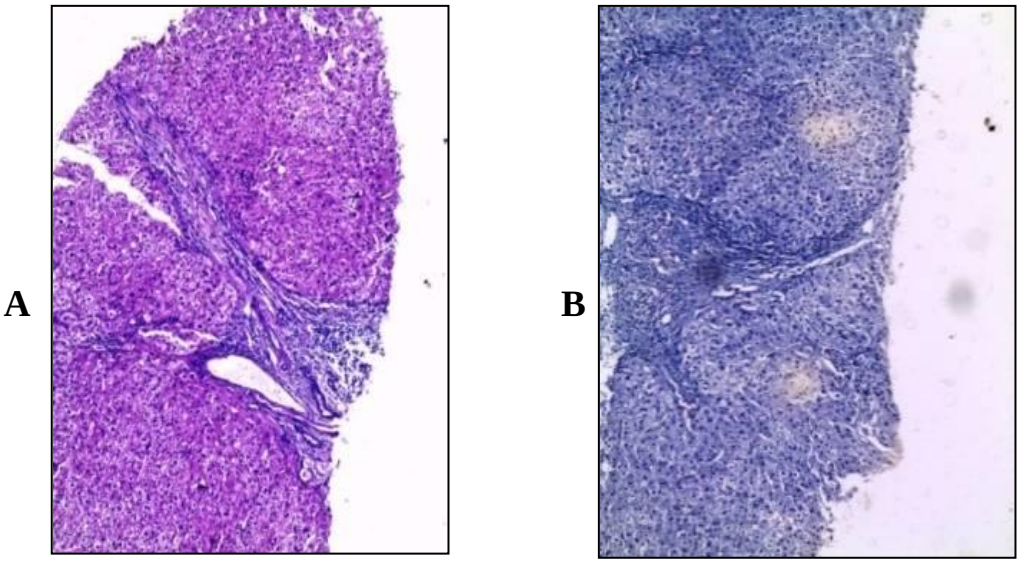

Fig. (1): Liver biopsy of chronic hepatitis B patient showing expansion of portal tract with fibrous septae (A1F1) using (H&E/100x) as in **(A)** & using masson's trichrome stain asin **(B)**

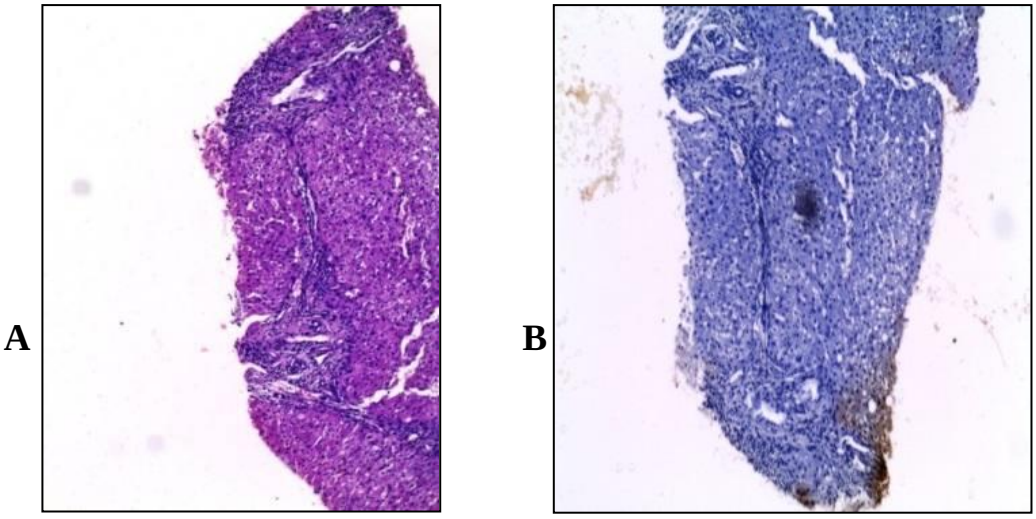

Fig. (2) : Liver biopsy of chronic hepatitis B patient showing bridging fibrous tissues (A2F2) using (H&E/100x) as in **(A)** & using masson's trichrome stain (100x) stain as in **(B)**

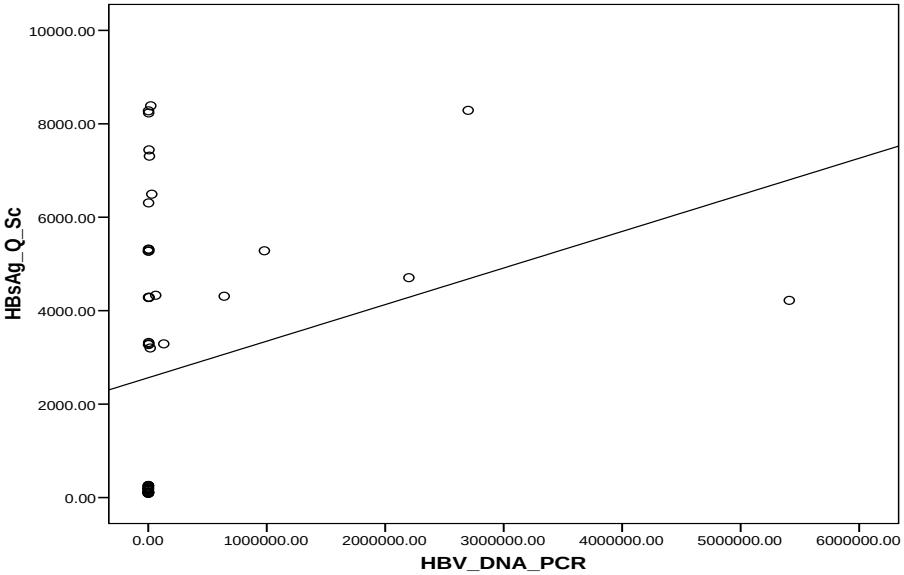

Fig. (3): Pearson correlation between HBV-DNA level and HBsAg Quantitation in all studied patients.

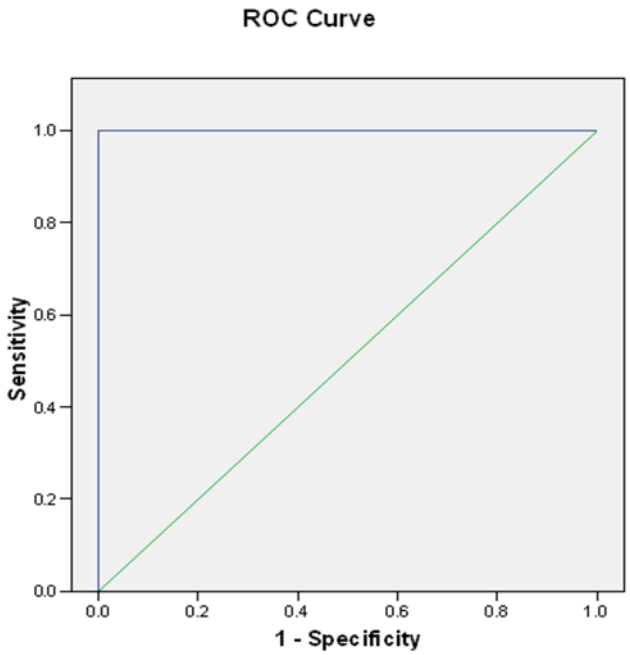

Fig. (4): The ROC (receiver operating characteristic) curve was for performance of HBsAg quantitation

DISCUSSION

The hepatitis B surface antigen (HBsAg) was identified more than 40 years ago. Detection of HBsAg in serum is still the hallmark of HBV infection and the cornerstone for the diagnosis of hepatitis B virus (HBV) infection. Over the years, HBsAg has been shown to be a reliable marker to predict clinical outcome **[8]**.

HBsAg is secreted into the circulation as tubular forms or spherical particles by HBV-infected hepatocytes. Patients with chronic hepatitis B (CHB) usually express different levels of HBsAg when assayed by immunohistochemistry **[9]**.

Recently, there has been an increasing focus on quantitative HBsAg (qHBsAg) and its use in the management of patients with CHB. The interest in qHBsAg started with the possible observation

of its correlation with intra-hepatic HBV covalently closed circular (ccc) DNA, a true marker of HBV replication **[10]**.

The quantitation of viral load is a routinely performed molecular test in clinical laboratories. The real-time PCR-based commercial assays for HBV DNA quantitation used in clinical practice have been available worldwide for several years **[11]**.

The gold standard tool in assessing the stage of liver fibrosis is the histological evaluation of a liver biopsy. However, the procedure carries a moderate risk of complications, including bleeding and a small risk of death **[12]**.

This study aimed to evaluate correlation between HBsAg quantitation assay, histopathology and HBV- DNA level in chronic HBV patients.

This study involved 50 patients with chronic hepatitis B; 37 of them were males (74%) and 13were females (26%). This finding goes in line with El- Zayadi et al. who reported that (46 male and 6 female) with a median age of 37.5 years in evaluation of Egyptian Chronic Hepatitis B Patients and HBeAg-negative variant accounts for more than 80 % of CHB in Egypt **[13]**.

In this study there is no correlation between complete blood count and liver profile (serum bilirubin, serum albumin, AST, alkaline phosphatase, α-fetoprotein and prothrombine time) with HBV-DNA levels, this is in agreement with Koyuncure, who concluded that there was no correlation between HBV-DNA levels and biochemical tests **[14]**.

In this study there is strong correlation among the studied groups between HBsAg quantitation and HBV-DNA levels (p=0.001), this finding is supported by other studies. For example, in the study by Alghamdi et al., study had found statistically significant positive correlation between HBsAg titers and HBV DNA levels **[15]**.

In addition Primadharsini and Wibawa **[16]** had showed significant and strong correlation between quantitative HBsAg and HBV-DNA. Furthermore the study by Hong et al.**[17]** who concluded that in the 183 patients in whom serum HBsAg levels were quantified, there was a positive correlation between HBsAg quantitation and HBV DNA levels (0.0001).

Also, Karagoz et al. had concluded that serum HBsAg titers correlate with HBV DNA in treatment-naïve patients **[18]**.

Another study by Ozgur et al. had reported that there was statistically significant correlation determined between HBsAg level and HBV DNA (P=0.0001) **[19]**.

Recently this also, had concluded by Gupta et al , who found that weak but significant correlation between quantitative HBsAg and HBV-DNA in all the groups (p<0.01) **[9]**.

Moreover, Li et al. had founded a significant positive correlation (r =0.642, P less than 0.0001) between HBsAg titre and HBV DNA load **[20]**.

In contrast with Ganiji et al. study who revealed that by electrochemiluminescence assay of HBsAg levels has no significant correlation with HBV DNA levels in chronic hepatitis B (p=0.606) **[21]**.

In addition, Mukherjee et al had founded that the correlation between serum HBV- DNA and serum concentration of HBsAg was not evident **[22]**.

Result in this study revealed that, there is no significant correlation between HBV-DNA levels and histopathology (fibrosis and activity) by Metaver score among the studied groups (p=0.467 & p=0.113 respectively). This finding is supported by other studies. For example, in the study by Shafaei et al. who revealed that there was no significant difference with fibrosis stage regarding different viral loads **[23]**.

In addition Xie et al. had observed that there is negative correlation between HBV DNA levels and significant fibrosis **[24]**. Furthermore Harkisoen et al. had revealed that there was no significant correlation between HBV DNA levels and liver fibrosis **[25]**.

In contrast to this study ; Bai et al. had found that positive correlation between the severity of hepatic inflammation and fibrosis and serum HBV DNA level (P < 0.001) **[26]**. This association was found by Praneenararat et al. who concluded that serum HBV-DNA level >5.5 log IU/ml should predict a significant liver fibrosis in treatment naïve chronic hepatitis B that had indicated for liver biopsy **[27]**.

In this study, there is statistically significant positive correlation among all studied groups between HBsAg quantitation and fibrosis (P= 0.018). This is in agreement with Cheng et al. who had concluded that the correlation between advanced liver fibrosis and HBsAg level was a significant **[28]**.

In addition to Zhong et al. who had concluded that the level of HBsAg of Chinese chronic hepatitis B patients correlate with liver inflammation and fibrosis **[29]**.

Furthermore, Hong et al. had found that there was a positive correlation between HBsAg levels and stage of fibrosis. Also, Marcellin et al. had concluded that there was significant correlation between HBsAg levels and advanced fibrosis **[30]**.

In this study, the validity of HBsAg titer in detecting cases with elevated HBV DNA level (≥ 2000 IU/ml) with HBsAg level at cut off point <1730.85 (IU/ml) with sensitivity, specificity (100% &100%, respectively) in all studied groups with P value <0.001 and Area under the curve (AUROC) = 1.

This study concluded that, there is strong significant correlation between HBsAg quantitation and HBV DNA levels, also there is correlation between HBsAg quantitation and fibrosis in contrast to no correlation between HBV DNA levels and histopathology ..

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Peer reviewer: Rashed Hasan, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor:Tarik Zaher ,** Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.