#### **Original Article**

# Viral Load Pattern Among Hepatitis B Surface Antigen-positive Patients: Laboratory Perspective and Implications for Therapy

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#### Abstract

**Background:** Hepatitis B viral infection is an old medical problem with worldwide distribution. It is usually diagnosed using serologic methods. However, the decision as to which patient to treat or not remains challenging due to the poor sensitivity of serologic markers as prognostic or severity markers. Viral load (VL) determination using polymerase chain reaction techniques is a useful tool in decision-making. Aim: To determine the proportion of hepatitis B-positive patients who fall into different care groups based on the Society for Gastroenterology and Hepatology in Nigeria (SOGHIN) and National Institute for Health and Care Excellence guidelines, respectively, using result of hepatitis B virus (HBV) DNA determination. Materials and Methods: This is a retrospective and descriptive study. Data from all patients sent to the medical microbiology laboratory, National Hospital Abuja over a period of 28 months (November 2012 to February 2015) for hepatitis B DNA VL determinations were analyzed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and IBM SPSS version 20.0 (IBM SPSS, Inc., Chicago, IL, USA). Results: A total 666 patients, with mean age of 33.2 years, were tested. For those whose ages were known 36.2% (100/276) were below 30 years and 63.8% (176/276) 30 years and above. Exactly 66.7% (444/666) were males and the remaining 33.3% (222/666) were females. The VL of the patients varied from 20 to  $1.7 \times 10^8$  IU/ml, with an average of  $3.5 \times 10^6$  IU/ml. Around 76.1% (507/666) had measurable assay levels (20 -  $1.7 \times 10^8$  IU/ml); 10.8% (76/666) had below 20 IU/ml and 3.8% (25/666) above  $1.7 \times 10^8$  IU/ml. About 9.3% (62/666) had no detectable HBV DNA in their samples. About 46.8% (312/666) of the patients had levels between 20 and  $2 \times 10^3$  IU/ml; 16.4% (109/666) had between 2001 and  $2 \times 10^4$  IU/ml while 16.7% (111/666) had VL of between 20,001 and  $1.7 \times 10^8$  IU/ml. Males tended to have detectable and higher VLs than females (P = 0.04). Conclusion: HBV DNA assay used in accordance with existing treatment guidelines will improve quality of care. To avoid unnecessary liver biopsy, there is a need to further fine-tune the SOGHIN guidelines.

Keywords: Hepatitis B, Surface antigen, Viral DNA assay

## Introduction

Over 2 billion people worldwide show some serological evidence of past or current hepatitis B virus (HBV) infection, of which 350 million are chronic carriers.<sup>[1-4]</sup> It is associated with bothersome sequelae.<sup>[3,5-7]</sup>

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West Africa has high endemicity rates particularly among infants due to vertical transmission.<sup>[8-11]</sup> In Nigeria, infection rate is between 7.3% and 24%.<sup>[1,12]</sup>

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How to cite this article: Iregbu KC, Nwajiobi-Princewill PI. Viral load pattern among hepatitis B surface antigen-positive patients: Laboratory perspective and implications for therapy. Ann Med Health Sci Res 2016;6:95-9.

Investigations for HBV infection include serologic assays for hepatitis B surface antigen (HBsAg), hepatitis B early antigen (HBeAg), antibodies to HBsAg, antibodies to HBeAg, antibodies to the hepatitis B core antigen.<sup>[2,5,13-15]</sup>

HbeAg, previously regarded as the best biomarker of HBV infectivity, is negative in a large population of HBV-infected individuals with detectable levels of HBV DNA.<sup>[16,17]</sup> Although HBV DNA levels correlate better with risk of developing liver complications, HBeAg is still considered in management decisions in some guidelines.<sup>[2,4,18-20]</sup>

Levels of HBV DNA above  $2 \times 10^3$  IU/ml are associated with significantly increased risk of liver cirrhosis and hepatocellular carcinoma.<sup>[4,5,7,21-25]</sup> Current guidelines provide treatment decision be based on the viral load (VL) level in conjunction with other parameters which include liver enzymes, age at presentation and liver histology. However, liver enzyme and histological findings do not always correlate.<sup>[4,5,15,26-29]</sup>

HBV DNA assay technology is still uncommonly used in our environment, essentially due to cost of equipment, test and need for high level trained manpower, and no evaluation of its suitability in the context of existing guidelines has been documented in our environment. This study was therefore designed to evaluate the profile of the assays using existing HBV treatment guidelines as reference.

## **Materials and Methods**

This was descriptive and retrospective. All patients referred for HBV DNA VL testing following a positive HBsAg screening test were included, while those without their gender and ages were excluded from the analysis. Where age was involved in the analysis, those without documented age were excluded from that very analysis. Only one HBV VL test carried out on a patient prior to any HBV targeted therapy was included in the study.

The laboratory records of all the 666 patients who met the inclusion criteria and had HB VLs test done from November 2012 to February 2015 were accessed for biodata (age, gender) and VL result. The data were analyzed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and IBM SPSS Version 20.0 (IBM SPSS, Inc., Chicago, IL, USA).

Sample processing for the HBV DNA VL determination was done in the polymerase chain reaction unit of the Department of Medical Microbiology and Parasitology, National Hospital Abuja. Patients' undergoing VL testing had 5 ml of venous blood collected from them into ethylenediaminetetraacetic acid containers, and then centrifuged at 3000 rpm at 9 g for 10 min. Plasma was separated and stored at  $-20^{\circ}$ C till further processing.

Stored samples were thawed to room temperature before 1000 µl of plasma was placed in the COBAS AmpliPrep (Roche

Molecular Systems, Inc., Branchburg, NJ, USA) for DNA extraction and priming. These were then transferred to the COBAS TaqMan48 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) for amplification and quantification. Results were measured in real time by the AMPLILINK ver. 3.3 software system (Roche Molecular Systems, Inc., Branchburg, NJ, USA). The system can accurately measure HBV assay levels  $20-1.7 \times 10^8$  IU/ml.

Ethical clearance was obtained from the Ethical Committee of the Hospital.

## Results

A total of 666 patients, of whom 66.7% (444/666) were males and 33.3% (222/666) females, were included in the study. Males had higher VLs than females (P = 0.04) [Table 1].

The ages of only 41.4% (276/666) were found documented, while the remaining 390 were documented as adults with no age specified. Of the 276 patients 36.2% (100/276) were below 30 years, while 63.8% (176/276) were 30 years and above [Table 2]. Those <30 years had higher VLs than those  $\geq$ 30 years (P < 0.01).

Around 76.1% (507/666) had measurable assay levels  $(20 - 1.7 \times 10^8 \text{ IU/ml})$ ; 10.8% (76/666) had below 20 IU/ml and 3.8% (25/666) above 1.7 × 10<sup>8</sup> IU/ml. Approximately 9.3% (62/666) had no detectable HBV DNA in their samples. Around 46.8% (312/666) of the patients had levels between 20 and 2 × 10<sup>3</sup> IU/ml; 16.4% (109/666) had between 2001 and 2.0 × 10<sup>4</sup> IU/ml while 16.7% (111/666) had VL of between 20,001 and 1.7 × 10<sup>8</sup> IU/ml [Table 3].

| Table 1: Association of viral load and gender |   |   |   |  |  |
|---|---|---|---|--|--|
| Gender  |   |   |   |  |  |
| Female  | Male  | OR  | 95% CI  |  |  |
| 26  | 36  | 1.000   | 0.883-2.560   |  |  |
| 29  | 43  | 0.934   | 0.849-2.313   |  |  |
| 102   | 210   | 0.673   | 0.688-1.368   |  |  |
| 32  | 77  | 0.573   | 0.513-1.256   |  |  |
| 33  | 78  | 0.586   | 0.526-1.276   |  |  |
| 222   | 444   | <i>P</i> =0.04  |   |  |  |
|   | <b>Female</b><br>26<br>29<br>102<br>32<br>33<br>222 | Female         Male           26         36           29         43           102         210           32         77           33         78           222         444 | Ciation of viral load and gender           Gender           Female         Male         OR           26         36         1.000           29         43         0.934           102         210         0.673           32         77         0.573           33         78         0.586           222         444         P=0.04 |  |  |

VL: Viral load, OR: Odds ratio, TND: Target not detected, CI: Confidence Interval

 Table 2: Distribution of hepatitis B viral load results into age categories used in some guidelines (n=276)

| VL  | Age cat   | Statistics |            |                |  |
|---|-----------|------------|------------|----------------|--|
|   | <30       | ≥30        | Total      |                |  |
| ND  | 5 (4.2)   | 18 (11.4)  | 23 (8.3)   | <i>P</i> <0.01 |  |
| <20   | 7 (7.0)   | 15 (8.5)   | 22 (7.8)   |                |  |
| 20-2×10 <sup>3</sup>  | 44 (44.0) | 77 (43.8)  | 121 (43.8) |                |  |
| 2001-2×104  | 13 (13.0) | 41 (23.3)  | 54 (19.6)  |                |  |
| >2×10 <sup>4</sup> ->1.7×10 <sup>8</sup>                                    | 31 (31.0) | 25 (14.2)  | 56 (20.3)  |                |  |
| Total   | 100 (100) | 176 (100)  | 276 (100)  |                |  |
| ND: HBV/ DNA not detected in sample HBV/: Hepatitis B virus VI : Viral load |           |            |            |                |  |

ND: HBV DNA not detected in sample, HBV: Hepatitis B virus, VL: Viral load

Among patients with measurable assays the youngest was 1 year old and the oldest 83 years, with an average age of 33.2 years. Mean assay level was  $3.5 \times 10^6$  IU/ml while the highest was  $1.5 \times 10^8$  IU/ml and the lowest 20 IU/ml. The modal class was 31–40 years [Table 4].

#### **Discussion**

There is a preponderance of males among HBV-infected persons with detectable VL as seen in this study. Although the reason is not clear, similar finding has been documented in previous studies.<sup>[30,31]</sup> Okwuraiwe *et al.*<sup>[11]</sup> had similar finding in Lagos and suggested it could be due to increased financial resources available to males to go for tests as against women. In contrast, Onwuliri *et al.*<sup>[31]</sup> and Okonko *et al.*<sup>[32]</sup> found more females with HBV infection among HIV patients and blood donors, respectively. A well-designed study may be needed to determine whether women abort the infection better than men.

The modal age range for measurable VL was 31-40 years, similar to 30-39 years obtained in Lagos<sup>[11]</sup> and 36-50 years in Bangladesh.<sup>[33]</sup> This may be related to the higher incidence of activities associated with HBV acquisition or reactivation of existing infections in this age group.<sup>[34,35]</sup> The highest mean was in the 1-10 year group, and was possibly associated with high perinatal transmission and a less competent immune status.<sup>[36]</sup> The net effect of this is that there were significantly higher VLs among subjects < 30 years, notwithstanding that the modal age for detection was in the 31-40 years modal group.

Effective management of HBV infection requires HBV DNA VL assay in accordance with existing treatment guidelines. A current guideline developed by the Society for Gastroenterology and Hepatology in Nigeria (SOGHIN) considers HBeAg status a major factor but discounts age.<sup>[13]</sup> In HbeAg-positive cases, the critical VL level is  $2.0 \times 10^4$  IU/ml. VL above this level with abnormal liver enzymes is an indication for chemotherapy, while

| Table 3: Profile of hepatitis B viral load results |            |  |  |
|--|------------|--|--|
| VL range   | Number (%) |  |  |
| ND   | 62 (9.3)   |  |  |
| <20  | 72 (10.8)  |  |  |
| 20-2×10 <sup>3</sup>                               | 312 (46.8) |  |  |
| 2001-2×10 <sup>4</sup>                             | 109 (16.4) |  |  |
| >2×10 <sup>4</sup> ->1.7×10 <sup>8</sup>           | 111 (16.7) |  |  |
| Total  | 666 (100)  |  |  |

ND: HBV DNA not detected in sample, HBV: Hepatitis B virus, VL: Viral load

VL  $<2.0 \times 10^4$  IU/ml with abnormal liver enzymes needs liver biopsy before chemotherapy can be considered. If patient is HbeAg negative, the critical VL level is  $2.0 \times 10^3$  IU/ml. A VL greater than this in combination with abnormal liver enzymes supports therapy, but if VL  $<2.0 \times 10^3$  IU/ml a liver histology is needed. Only in the presence of moderate to severe fibrosis is chemotherapy indicated.

Under the SOGHIN guidelines, assuming all patients were HBeAg-positive with an abnormal alanine aminotransferase (ALT) level, 74.0% who have VL <2.0 × 10<sup>4</sup> IU/ml would need a liver biopsy for further assessment while 16.7% would qualify for chemotherapy based on their DNA and abnormal ALT alone. On the other hand if it is taken that all the patients were HBeAg negative with abnormal ALT levels, then 33.1% would qualify for chemotherapy while 57.6% would need a liver biopsy for determination of appropriate therapy. Therefore, it follows that a large number of patients would be subjected to liver biopsy with its attendant risk.<sup>[37]</sup> This would appear to be a challenge in using the SOGHIN guidelines despite its advantage that a single VL and liver function tests could be used to determine therapy.

The National Institute for Health and Care Excellence (NICE) guidelines recognize VL values of  $2.0 \times 10^3$ – $2.0 \times 10^4$  IU/ml as critical cutoff points when considering therapy, in conjunction with age, ALT levels, pregnancy/breastfeeding, and liver histology.<sup>[22]</sup> In patients aged  $\geq 30$  years, with VL> $2.0 \times 10^3$  IU/ml and ALT > 30 IU/l (male) or > 19 IU/l (females) on two consecutive occasions at least 3 months apart chemotherapy is indicated. However, when the patient is < 30 years with similar findings, an abnormal liver biopsy is needed before considering chemotherapy. In cases where VL  $> 2.0 \times 10^4$  IU/ml with abnormal ALT levels, then chemotherapy is indicated. Cases of active liver disease with VL  $> 2.0 \times 10^3$  IU/ml or cirrhosis with any VL level also require therapy.

The slightly different approach followed by the NICE guidelines means that if all patients' ALT levels are taken as abnormal, then 23.3% would be placed on chemotherapy among those aged  $\geq$  30 years while 13.0% of those aged < 30 years would need a liver biopsy for further assessment and subsequent management. 20.3% would be eligible regardless of age for chemotherapy since their VL is  $> 2.0 \times 10^4$  IU/ml. In effect the NICE guidelines may be associated with fewer liver biopsies.

The highest measurable VL load range was recorded in the 1-10 years age group. This is where the widest variation

| Table 4: Distribution of measurable viral load parameters by age groups ( <i>n</i> =276) |                        |                        |                     |                     |                     |                         |                        |  |
|--|------------------------|------------------------|---------------------|---------------------|---------------------|-------------------------|------------------------|--|
| Mean VL  | Age groups             |                        |                     |                     |                     |                         |                        |  |
|  | 1-10                   | 11-20                  | 21-30               | 31-40               | 41-50               | 51-60                   | >60                    |  |
|  | 3.6×10 <sup>7</sup>    | 1.7×10 <sup>7</sup>    | 2.4×10 <sup>6</sup> | 2.1×10 <sup>6</sup> | 6.2×10 <sup>6</sup> | 2.8×10⁵                 | 632                    |  |
| Viral load range   | 48-1.5×10 <sup>8</sup> | 20-1.3×10 <sup>8</sup> | 26-6.8×107          | 28-7.4×107          | 21-5.6×107          | 124-3.9×10 <sup>6</sup> | 63-2.1×10 <sup>3</sup> |  |
| Number of patients   | 15                     | 38                     | 65                  | 79                  | 58                  | 15                      | 6                      |  |
| VL: Viral load   |                        |                        |                     |                     |                     |                         |                        |  |

Annals of Medical and Health Sciences Research | Mar-Apr 2016 | Vol 6 | Issue 2 |

occurred. This can be explained by the relatively naïve immune system in children and the different clinical course of the infection in this age group.<sup>[33,36,38]</sup> This emphasizes the need for both maternal and childhood vaccination against HBV infection.<sup>[10,27,36,38]</sup> The retrospective nature of this work is a major limitation, however the information to the scientific community is very relevant to patient management.

#### Conclusion

VL testing is important in making management decisions in HBV infection. It will help to avoid unnecessary therapies, commence treatment as appropriate, and save cost. More research is needed to further fine-tune the local guidelines. Access to HBV DNA assay needs to be increased through some kind of support to enhance quality of care and research.

# Financial support and sponsorship

Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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