Profiling Hepatitis B Viral Load: Treatment and Epidemiological Implications in a West African Hospital

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Abstract

Background: Chronic hepatitis B virus (HBV) infection is one of the largest public health problems with nearly 350 million chronic carriers and 500,000 deaths each year. These deaths are most often associated with disease progression to cirrhosis or hepatocellular carcinoma, which some studies have shown is associated with long-term viral replication in chronic carriers. Viral load quantification, a key element of disease management, is expensive and difficult to access. Viral load plays a crucial role in patient classification and treatment initiation. Four years after the implementation of viral load platform, the objective of this study was to assess viral load profile in HBs chronic carriers in a sub-Saharan Hospital and to determine the potential impact of this distribution on preventive and therapeutic strategies against hepatitis B infection. Materials and Method: The study was carried out between April 2016 and October 2020 in the laboratory of the PRINCIPAL Hospital in Dakar. All patients referred for HBV DNA viral load testing following a positive AgHBs test were included. Incomplete medical records were excluded from the study. Only the first quantification test performed on each patient is recorded. DNA extraction was performed with COBAS Amplicor (Roche Molecular Systems, Inc., Branchburg, NJ, USA). Amplification was performed using COBAS TaqMan48 (Roche Molecular Systems, Inc., Branchburg, NJ, USA). Data were collected from the laboratory's computer system and entered into Microsoft Excel (2007). Statistical analyzes were performed using...
Epi-Info 7 software. **Results:** A total of 3002 patients, 76.1% (2285/3002) men and 33.9% (717/3002) women, were included in the study. Young adults were most represented among the subjects (23.2%) and (20.1%) in the age groups 25 - 30 and 30 - 35. The majority (52.7%) of patients had viral loads between 20 and 2000 IU. Patients with undetectable viral loads and patients with viral loads below 20 IU comprised 14.6% and 7.53% of the study population, respectively. Patients with viral loads between 2000 and 20,000 IU/ml and those with viral loads greater than 20,000 IU/ml represented 16.3% and 8.89% of the study population, respectively. Viral load was higher in males than females, with corresponding median and interquartile ranges of 2.7 log IU (2.2, 2.75) and 2.23 log IU (2.1, 2.4) (p < 0.001). This viral load also decreases with age. There were more patients with an undetectable viral load over 30 years than those under 30 years, with a ratio of 1.62 (IC95% = 1.29 - 2.05) (p = 0.009). **Conclusion:** This study shows a successful implementation of virus quantification in the context of resource-constrained countries. The second finding of this study is the high prevalence of adolescents with high plasma viral loads, indicating the need for additional investigations to initiate therapy. The large population with a low HBV replication rate points to the problem of financing follow-up care for chronically infected people. Studying this population in the context of an unknown genomic profile indicates the need to deepen virological laboratory testing through a sequencing platform. Finally, regular viral load reporting in major hospital cities could be a powerful and accessible management tool for hepatitis B programs in resource-constrained countries.

**Keywords**
Hepatitis B, Viral Load, Senegal, Treatment

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1. **Introduction**

Chronic hepatitis B virus (HBV) infection is a major public health problem, causing nearly 350 million chronic carriers and 500,000 deaths each year. Mortality is most commonly associated with disease progression to cirrhosis or hepatocellular carcinoma, which has been linked to prolonged viral replication in chronic carriers in some studies. Only 9% of those infected with HBV (22 million) were aware of their condition, and treatment faces several challenges, including surveillance [1]. Laboratory tests, which are a key component of both diagnosis and follow-up, are expensive and not widely available. Unfortunately, viral load quantification is not possible in many laboratories in Senegal due to a lack of funds. Among the few laboratories that perform tests, the cost is high ($60 - $200/test) due to the price of the reagents [2]. In remote areas, the lack of skilled personnel, the cost of equipment and reagents, and the challenges of maintaining the cold chain make HBV quantification difficult. Very often, patients have to travel long distances to visit laboratories, usually located in urban centers, to have the viral load quantification test performed. Overall, it is estimated that between 10% and 40% of HBV-positive individuals require hepatitis
This treatment is generally recommended for all patients with cirrhosis based on clinical assessment and biological testing. In patients without cirrhosis, assessment of treatment appropriateness and response requires access to quantitative measurements of HBV viral DNA. Treatment of cirrhosis-free patients is generally recommended in most international guidelines for individuals with HBV DNA levels above 2000 IU/mL and abnormal liver function tests. One of the major challenges in planning the resources needed to treat HBV in sub-Saharan Africa is the lack of available information on the proportion of the HBV-infected population that is eligible for antiviral treatment. As TDF becomes more accessible to the Senegalese population, there is an urgent need to know the treatment needs in Senegal. The viral load thus plays a fundamental role in the classification of patients with regard to the course of the disease and the need for treatment. In this context, our aim was to determine the HBV viral load profiles observed in chronic HBV carriers who performed their testing at the Principal hospital in Dakar. Secondly, based on the different observed viral load profiles, discuss the usefulness of sentinel monitoring based on regular assessment of viral load profiles. The main expectations for these results would be, firstly, a better understanding of people with chronic hepatitis B infection by analyzing the different patterns of viral load observed. Secondly, based on the different viral load profiles observed, discuss the urgent need for sufficient drugs and complementary laboratory tests such as a sequencing platform. The ultimate goal is to discuss the usefulness of regular viral load assessment as indicators that, despite their limitations, help predict the need for supplemental laboratory testing (sequencing platform) and treatment for patients given the complex nature of hepatitis B virus infection.

2. Method

2.1. Study Area Description

The Principal Hospital of Dakar (HPD) is located in the Dakar Plateau district of Senegal. It is a public health facility with military status. Principal is a 471-bed Level 3 hospital with a large hepatology department monitoring a high proportion of Senegalese hepatitis B carriers (Figure 1).

![Figure 1. The Dakar hospital principal (HPD).](image-url)
2.2. Study Design

This is a retrospective cross-sectional study conducted between April 2016 and October 2020 at the Federation of Laboratories of the PRINCIPAL HOSPITAL OF DAKAR. All patients who were referred for HBV DNA quantification testing following a positive HBsAg (hepatitis B surface antigen) result were included. Records with missing information were excluded from the study. Only the initial viral load quantification test was selected for each patient.

2.3. Data Collection and Analysis

Viral load age and sex information was collected for all patients via the laboratory's computer system. Data entry and statistical analysis were performed with Epi-info version 7. Means and their standard deviations as well as medians and their interquartile ranges (IQR) were calculated for the quantitative variables. The proportions of qualitative variables were determined with a confidence interval (CI) of 95%. Outcome variables were viral load status. Cross-tabulations were created from the result variables and the socio-demographic variables (age, gender). Chi-square or Fisher’s exact test was used as a statistical significance test to compare sociodemographic variables with viral load status. A p-value < 0.05 from two-tailed tests was considered significant in all tests. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine associations between sociodemographic variables and viral load status.

2.4. Inclusion and Exclusion Criteria

2.4.1. Inclusion Criteria
All patients referred after a positive HBsAg (hepatitis B surface antigen) result for an HBV DNA quantification test were included.

2.4.2. Exclusion Criteria
All patients whose medical records were not traceable were excluded from the study.

2.5. HBV Viral Load Quantification

Two EDTA tubes containing 5 ml of venous blood were taken from each patient and then centrifuged at 9 g and 3000 rpm for 10 min. For patients whose testing was delayed, plasma was separated and stored at −20°C until treatment. Stored samples are thawed at room temperature and 1000 μL of plasma per patient is added to COBAS AmpliPrep (Roche Molecular Systems, Inc., Branchburg, NJ) for DNA extraction. In the last phase of the extraction, the DNA is mixed with the reaction mixture containing the polymerase. The reaction extract mixture is then transferred to COBAS TaqMan48 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) for amplification and quantification. Results are reported in real time by AMPLILINK ver. 3.3 software system (Roche Molecular Systems, Inc., Branchburg, NJ, USA). The system can accurately measure HBV viral loads ranging from 20 to $1.7 \times 10^8$ IU/ml.
3. Results

3.1. Study Population

A total of 3002 patients, 76.1% (2285/3002) males and 33.9% (717/3002) females, were included in the study. In the 20 to 25 age group, 83.5% are men. Compared to the 30 - 35 age group, there are 1.57 times more men in the 20 - 25 age group. Overall, more men are under 30 than over 30 years old. The young adults were most strongly represented with (23.2%) and (20.1%) of the subjects in the respective age groups 25 - 30 and 30 - 35 years. The under-20s were the least represented at 1.60% of the population surveyed (Table 1).

3.2. Viral load Distribution in the Study

The majority (52.7%) of patients had viral loads below 2000 IU. Subjects with an undetectable viral load and subjects with viral loads below 20 IU comprised 7.53% and 14.6% of the study population, respectively. Subjects with viral loads between 2000 and 20,000 IU/mL and subjects with viral loads greater than 20,000 IU/mL represented 16.3% and 8.89% of the study population, respectively (Table 2).

3.2.1. Viral Load Distribution and Gender

A significant association was found between hepatitis B viral load and gender. The median viral load was higher in men (2.70 log IU [P25: 2.64; P75: 2.75]) than in women (2.23 log IU [P25: 2.10; P25: 2.40]) with an odds ratio of odds ratio = 1.26 (IC95% = 1.19 - 1.34 p < 0.001) and males are more likely to have a high viral load than females, with a ratio of 4.76 in patients with viral loads above 20,000 IU/mL (Table 2).

### Table 1. Characteristics of study population.

| Age, Median [25th; 75th] | [ALL] N = 3002 | Women N = 717 (23.9%) | Men N = 2285 (76.1%) | OR | p.ratio | p.overall |
|--------------------------|----------------|----------------------|---------------------|----|---------|-----------|
| <30 years old            | 33.0 [33.0; 34.0] | 35.0 [34.0; 36.0] | 33.0 [32.0; 34.0] | 0.99 | [0.98; 0.99] | <0.001 |<0.001 |
| >30 years                | 64.5%           | 25.6% [23.6%; 27.6%] | 74.4% [72.4%; 76.4%] | 0.78 | [0.65; 0.94] | 0.008 |

| Age group, N (%)         | <0.001 |<0.001 |
|--------------------------|--------|--------|
| <20                      | 1.60%  | 50.0% [35.2%; 64.8%] | 50.0% [35.2%; 64.8%] | 0.31 | [0.17; 0.57] | <0.001 |
| 20 - 25                  | 9.09%  | 16.5% [12.3%; 21.4%] | 83.5% [78.6%; 87.7%] | 1.57 | [1.09; 2.30] | 0.014 |
| 25 - 30                  | 23.2%  | 21.0% [18.0%; 24.2%] | 79.0% [75.8%; 82.0%] | 1.17 | [0.90; 1.52] | 0.237 |
| 30 - 35                  | 20.1%  | 23.8% [20.4%; 27.4%] | 76.2% [72.6%; 79.6%] | Ref. | Ref. |
| 35 - 40                  | 13.1%  | 24.1% [20.0%; 28.6%] | 75.9% [71.4%; 80.0%] | 0.98 | [0.73; 1.32] | 0.895 |
| 40 - 45                  | 11.1%  | 22.9% [18.5%; 27.8%] | 77.1% [72.2%; 81.5%] | 1.05 | [0.76; 1.45] | 0.769 |
| 45 - 50                  | 7.89%  | 23.6% [18.4%; 29.6%] | 76.4% [70.4%; 81.6%] | 1.01 | [0.71; 1.44] | 0.975 |
| >50                      | 14.0%  | 31.4% [26.9%; 36.0%] | 68.6% [64.0%; 73.1%] | 0.68 | [0.52; 0.90] | 0.007 |

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Table 2. Viral load distribution and gender.

| Viral loads (Log UI)        | Women  | Men         | OR   | p.ratio | p.overall |
|----------------------------|--------|-------------|------|---------|-----------|
| Median [25th; 75th]        | N = 717 (23.9%) | N = 2285 (76.1%) |      |         |           |
| 2.60 [2.53; 2.65]          | 2.23 [2.10; 2.40] | 2.70 [2.64; 2.75] | 1.26 [1.19; 1.34] | <0.001 | <0.001 |
| Viral loads (UI) Median [25th; 75th] | 381 [331; 439] | 169 [126; 232] | 492 [429; 552] | 1.00 [1.00; 1.00] | 0.017 <0.001 |

Viral load category, N (%): <0.001

| Category        | Women  | Men         | OR   | p.ratio | p.overall |
|-----------------|--------|-------------|------|---------|-----------|
| 20 - 2000 UI    | 52.7%  | 21.9% [19.9%; 24.0%] | 78.1% [76.0%; 80.1%] | 1.92 [1.53; 2.42] | <0.001 |
| 2000 - 20,000 UI| 16.3%  | 24.7% [20.9%; 28.8%] | 75.3% [71.2%; 79.1%] | 1.64 [1.24; 2.18] | 0.001 |
| undetectable    | 14.6%  | 35.0% [30.5%; 39.7%] | 65.0% [60.3%; 69.5%] | Ref. Ref. | Ref. |
| <20 UI          | 7.53%  | 31.0% [25.0%; 37.4%] | 69.0% [62.6%; 75.0%] | 1.20 [0.85; 1.70] | 0.299 |
| >20,000 UI      | 8.89%  | 10.1% [6.77%; 14.4%] | 89.9% [85.6%; 93.2%] | 4.76 [3.10; 7.56] | <0.001 |

3.2.2. Viral Load Distribution and Age

Conversely, the connection between age and viral load is established, the viral load decreases with age. High viral loads are observed in younger subjects with a mean age of 34 years in the category of subjects with viral loads over 20,000. The mean age in the group with no detectable viral load is 41 years, which is 7 years older than in the group with more than 20,000 IU/mL viral load (Table 3). Those over 30 years of age account for almost three-quarters (73%) of the undetectable group, while in the group with a viral load above 20,000 IU/mL, those over 30 years of age account for only 53% (Table 3).

4. Discussion

4.1. Viral Load Implementation Challenges

Four years after the implementation of the hepatitis B virus quantification platform, 3002 subjects were enrolled in the Principal hospital laboratory. One of the early achievements was the ability to deploy the technology locally, allowing for more accurate patient follow-up planning. The second point was to reduce the additional costs associated with contracting foreign laboratories to perform viral load quantification. For a patient paying at their own expense, this saves both time and subsidizes the cost of $60 to $200 per viral load test performed [5]. If you add all the biological tests for hepatitis B, the level of the fees is not far from the average civil servant salary in Congo [2]. Viral load testing is therefore a significant source of non-compliance with follow-up among chronic hepatitis B carriers. In terms of logistics, the automated extraction of our platform significantly reduces contamination. Second, most reagents do not require harsh storage conditions, with temperatures typically around 4 degrees Celsius. The cold chain issue is a key element in conducting viral load testing in sub-Saharan Africa, where temperatures are often high.
Table 3. Viral load distribution and age.

| Age category     | N (x1000) | <20 IU N = 226 | >20,000 IU N = 267 | p.overall |
|------------------|-----------|----------------|---------------------|-----------|
| <0.001           |           |                |                     |           |
| Age mean (SD)    |           |                |                     |           |
| <20              | 1.60% [1.18%; 2.11%] | 0.57% [0.26%; 1.08%] | 1.84% [0.84%; 3.46%] | 3.89% [2.28%; 6.16%] | 3.10% [1.25%; 6.28%] | 2.25% [0.83%; 4.83%] |
| 20 - 25          | 9.09% [8.09%; 10.2%] | 9.67% [8.26%; 11.2%] | 9.80% [7.31%; 12.8%] | 7.09% [4.87%; 9.92%] | 5.31% [2.77%; 9.09%] | 10.9% [7.40%; 15.2%] |
| 25 - 30          | 23.2% [21.7%; 24.7%] | 24.4% [22.3%; 26.6%] | 25.7% [21.9%; 29.8%] | 14.6% [11.5%; 18.3%] | 15.0% [10.6%; 20.4%] | 31.8% [26.3%; 37.8%] |
| 30 - 35          | 20.1% [18.6%; 21.5%] | 22.2% [20.2%; 24.3%] | 23.5% [19.8%; 27.5%] | 11.9% [9.02%; 15.3%] | 18.1% [13.3%; 23.8%] | 16.1% [11.9%; 21.1%] |
| 35 - 40          | 13.1% [11.9%; 14.4%] | 12.5% [10.9%; 14.2%] | 13.5% [10.6%; 16.8%] | 13.5% [10.4%; 17.1%] | 16.4% [11.8%; 21.9%] | 12.7% [8.98%; 17.3%] |
| 40 - 45          | 11.1% [9.96%; 12.2%] | 10.9% [9.44%; 12.6%] | 10.6% [8.03%; 13.7%] | 10.5% [7.81%; 13.8%] | 13.7% [9.51%; 18.9%] | 11.2% [7.71%; 15.7%] |
| 45 - 50          | 7.89% [6.95%; 8.92%] | 7.84% [6.56%; 9.27%] | 6.53% [4.51%; 9.09%] | 8.92% [6.42%; 12.0%] | 10.6% [6.92%; 15.4%] | 6.74% [4.04%; 10.4%] |
| 50 plus          | 14.0% [12.8%; 15.3%] | 11.9% [10.3%; 13.6%] | 8.57% [6.25%; 11.4%] | 29.5% [25.3%; 34.0%] | 17.7% [13.0%; 23.3%] | 8.24% [5.24%; 12.2%] |

4.2. A Globally Low Level of Replication

The majority, 1582 of our study population (52%), consists of subjects with low HBV replication; Viral load below 2000 IU/ml. In 2006, a molecular epidemiological study conducted in Principal hospital showed in blood donors and chronic hepatitis B a low viral load and the patients were mainly infected with genotype E (72%). Patients infected with genotype A (28%) tended to be younger than other patients, suggesting a potential influence of sex pathway on genomic diversity [6]. Studies in children showed the same mixed genome pattern with (53%) belonging to genotype A and (47%) to genotype E [7]. A recent study by Jaquet et al. showed that almost half of the patients had an A genotype [8] and patients with genotype E were more likely to be candidates for anti-HBV treatment than patients with genotype A. In several studies including Bujumbura, East Asia [5] [9] and many other countries, the hepatitis B virus genotype showed a replication pattern that has been reported at low levels. Similar results were reported in Nigeria by Lesi et al. This low-level replication pattern has been described by some authors as characteristic of genotype A. This genotype is characterized by an early clearance of AgHBe, the prevalence of which does not exceed 20% to 40% in adults [10], a low viral load does not always mean safety, according to Nicola Coppola’s study of 116 migrants. Of the 35 subjects with serum HBV DNA of 2000 IU/ml, 2.9% had chronic hepatitis and 2.9% had liver cirrhosis liver [11]. It is very likely that the current guidelines do not take into account chronic hepatitis HBV genotype E, a genotype that has been detected mainly in SSA populations in recent years. Therefore, the molecular epidemiology of HBV distribution largely supports the low viral load in the study population. The importance of this category of low replication subjects (52% of the
study population) reflects the medium-term need for the implementation of patient genotyping in Senegal. In fact, several authors reported that the evolutionary profiles of chronic HBV carriers, as well as viral mutations and anti-HBe seroconversion, are highly dependent on genotypes.

4.3. Approximately 10% above the WHO Inclusion Limit for Viral Load of 20,000 IU/ml for Therapy

4.3.1. Elevated Viral Load in Men
Almost 90% of people with viral loads above 20,000 IU/mL in our study are men. On the other hand, the proportion of subjects with an undetectable viral load was higher in females than in males, with a ratio close to 2. Higher viral loads in male subjects have been reported by several authors [4] [12], including Getinet Ayano et al. to a meta-analysis [13]. For some authors, economic reasons are very important [14]. In fact, in many African countries, men have higher financial incomes and are more willing to undergo screening and follow-up testing for the disease [4] as in our study. Male mobility in countries with limited resources is therefore considered by several authors to be a risk factor for the sexual transmission of hepatitis B. Sex hormones such as androgens and estrogens have been shown to play very different roles in the progression of HBV infection and in the development of HCC associated with HBV. The mechanism of gender disparity is thought to be related in part to the rather synergistic effects of female sex hormones on the immune response, regulation of HBx transactivation, and release of inflammatory cytokines [15]. However, further studies are needed for a better understanding of the mechanism.

4.3.2. Increased Viral Load in Young People
About 46.5% of people with a viral load above 20,000 IU/mL in our study are under 30 years old. At 31.8%, the 25- to 30-year-old age group accounts for almost a third of people with a viral load above 20,000 IU/ml. Overall, viral load is significantly dependent on age, with a mean of 34 years for patients with a viral load above 20,000 IU/mL compared to 41 years for patients with an undetectable viral load. Similarly, people over 30 had a higher propensity to be undetectable (ratio 1.62) than people under 30. The same results were reported in Lagos [16] and Bangladesh. Several studies have shown that young age is a risk factor for acquiring and reactivation of infections such as hepatitis B, particularly via sexual routes [5]. High viral loads have been described in The Gambia and Taiwan as important risk factors for the development of hepatocellular carcinoma or liver cirrhosis, regardless of antigenic HBe status. An HBV viral load above 20,000 IU/ml was a strong predictor of advanced liver disease in the Aberra cohort in Addis Ababa [17]. Several studies, including the REVEAL group study, have shown a high risk of developing hepatocellular carcinoma in individuals with a high persistent viral load [18] [19]. For some authors, the risk of hepatocellular carcinoma starts to increase significantly at a serum HBV-DNA level of 10,000 copies/ml. Therefore, many clinical practice guidelines suggest making
management decisions in the treatment of patients with chronic hepatitis B when viral load reaches this level [18] [20]. Recent data from countries where HBV is less endemic suggest that only a small proportion of people with chronic HBV infection who are candidates for antiviral therapy have access to it [21]. In resource-constrained countries, the persistence of high viremia often goes unnoticed due to a lack of means to quantify viral load. In our study, those under 30 years old with a viral load above 20,000 IU/mL make up 43% of this group. At this age, most young people do not have the financial means to see a doctor, which poses a significant health risk. This group is both medically and epidemiologically sensitive due to the increased risk of infection transmission and carcinoma development. Comprehensive and quantitative analysis of this high viral load group is therefore an important tool to help national programs identify primary hepatitis B treatment needs. Most of these needs relate to the availability of drugs, laboratory tests, and imaging tests that are needed immediately when viral loads are high.

4.4. Fifth Study Population under the Viral Load Detection Limit (20 IU)

A total of 437 patients had undetectable viral loads and 226 had viral loads below 20 IU, for a total of 663 below the detection limit. Patients below the threshold of detection account for 22% (IC95% = 20.6 - 23.6) of the study population. Similar rates were obtained in Nigeria, where 10.8% had viral loads below threshold and 9.3% had undetectable viral loads, for a total of 20.1% below the 20 IU/L threshold. This population often includes patients who are treated and are successful with their therapies. The WHO estimated that of the 257 million people living with chronic hepatitis B virus (HBV) infection worldwide in 2016, only 10.5% had been diagnosed and of these only 17% were treated [22]. According to Vu & Col, approximately 40% of eligible patients have not started treatment after a longer follow-up [3] [21]. Therefore, regular assessment of the group of undetectable viral loads is an indirect indicator of the chronically infected population in which viraemia is controlled. Changing proportions of this population can therefore be a good indicator of management efficiency. This indicator will have its limitations, including a lack of information on clinical and biochemical evaluation. However, it will have the advantage of being available in all major hospitals across the country with a viral quantification platform.

5. Conclusion

This study demonstrates a good implementation of virus quantification in the context of resource-constrained countries. After four years of practice, the activity has become routine, with increasing demand reflecting the reality of endemic HBV infection. The second finding of this study is the high prevalence of adolescents with high plasma viral loads, indicating the need for further investigation. This group is very interesting from an epidemiological point of view since
it is the bed of sexual transmission. Our data points out the need to improve vaccination practices and the implementation of early therapies in those at risk. The population with a low HBV replication rate was also well represented. Due to its importance, this population points to the problem of funding the follow-up of chronically infected people and the additional funds to deepen virologic laboratory tests through additional means such as a sequencing platform. Finally, regular viral load reporting in major hospital cities could be a powerful and accessible tool for hepatitis B programs in resource-constrained countries.

Authors' Contributions

All authors were involved in data collection and the preparation of the final manuscript

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Conflicts of Interest

The authors declare that they have no competing interests.

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**List of Abbreviations**

HBsAg: hepatitis B surface antigen; HPD: Principal Hospital of Dakar; WHO: World health organization