

Serological Marker Profiles of Chronic Hepatitis B Virus Infection among HBsAg-Positive Patients Seeking Medical Care in the North Rift Region of Kenya

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Abstract

Background: Serological marker and viral load testing in hepatitis B virus (HBV) infection is essential for accurate diagnosis, interpretation of serological profiles and infectivity, guiding treatment, and monitoring patient progress. In Baringo County, Kenya, the HBV prevalence of 10.8%, exceeds the national average of 3%, indicating a hyperendemic focus. Despite the adoption of World Health Organization (WHO) HBV treatment guidelines at Moi Teaching and Referral Hospital and Marigat Sub-County Hospital in 2017, routine use of serological markers and viral load testing remains limited in clinical practice. **Methods:** This cross-sectional study included 65 chronic HBV patients receiving treatment at Moi Teaching and Referral Hospital and Marigat Sub-County Hospital. Plasma samples were analyzed for HBsAg, HBsAb, HBeAg, HBeAb and HBcAb-IgM using the Quick Profile™ HBV-5 panel Test. HBV DNA viral load was quantified by quantitative real-time polymerase chain reaction (qPCR). Descriptive statistics were used to summarize the distribution of serological markers, while viral load results were expressed as medians with interquartile ranges (IQR). The Chi-square test was used to assess categorical distributions. **Results:** All 65 (100%) subjects were found to have HBsAg, confirming chronic HBV infection. The most frequent pattern was HBsAg (+), HBsAb (–), HBeAg (–), HBeAb (–), HBcAb-IgM (–) and was seen in 31 patients (47.7%). This profile was typical of chronic Hepatitis B infection with no evidence of acute activity or HBeAg seroconversion in the last several months. HBV DNA levels were relatively low in this group with a me-

dian of 509 copies/mL (IQR: 28.7 - 14,231) with some variation in viral load seen in many individuals, mostly low-replicative. Twenty-three-point one percent (23.1%) of patients were HBcAb-IgM positive, which is an active immune response and suggests viral reactivation. HBeAb positivity without HbcAb-IgM was detected in 21.5%, which is in line with HBeAg-negative serological profile with persistent viral replication. 7.7% of participants were co-positive for HBsAb and HBsAg, indicating partial immune control. The median viral loads were significantly different between serological profile: lowest for HBcAb-IgM positive patients, 162 copies/mL (IQR: 28 - 1,500,000); highest for HBeAb positive without HBcAb-IgM, 17,589 copies/mL (IQR: 118 - 27,353,162); and intermediate for those with both HBsAb and HBsAg positivity, (1642 copies/mL IQR: 567 - 11,466). **Conclusion:** The serological and viral load profile were very diverse, indicating different serological patterns and immune responses within this hyperendemic population. Regularly performed serological and viral load assays are important for proper disease staging and management. Improving the diagnostic capacity will allow for targeted treatment and augment public health interventions.

Keywords

Hepatitis B Virus, Chronic Infection, Viral Load, Tenofovir, Entecavir, Serological Markers

1. Introduction

Chronic Hepatitis B virus (HBV) infection is one of the most significant public health problems around the world, where 254 million people are living with the infection and 1.1 million people died from the infection in 2022, mainly from liver cirrhosis and hepatocellular carcinoma [1]. Approximately 65% of HBV infections worldwide are due to perinatal and early childhood horizontal transmission [2]. The prevalence of HBV infection among people aged 15 - 64 years in Kenya is estimated at 3% and is higher in northern and western and other resource-limited areas of Kenya [3]. A recent systematic review and meta-analysis found a national prevalence of 7.8%, with significant regional and population differences [4]. Marigat Sub-County in Baringo County, Kenya, in the Rift Valley region is reported to have a relatively high HBV infection burden [5]. The prevalence of HBV (6.3%) in pregnant women [6] was reported in a study conducted at Marigat Sub-County hospital indicating that there is still community-level transmission of the disease. Although the burden is high, the local health facilities lack diagnostic capacity and patient monitoring, which are limiting the effective management of hepatitis B, and leading to sustained transmission and progression to more advanced liver disease.

Chronic HBV infection is a dynamic, complicated process, in which viral replication and host immune responses interact [7]. There are different stages of the infection, which are characterised by different viral replication, expression of viral

antigens and hepatic inflammation: immune-tolerant phase, immune-active phase, inactive carrier (immune control) and HBeAg-negative chronic hepatitis (reactivation phase) [8]. During the immune-tolerant phase, the individual has high levels of HBV DNA, is HBeAg-positive, and has little liver inflammation [8] [9]. During the immune-active phase, there is increased hepatocyte injury caused by immune mechanisms, varying levels of HBV DNA, and progressive liver injury which can result in liver fibrosis [10]. In the low-replicative serological profile, patients continue to be HBsAg positive, but are HBeAg negative or have anti-HBe antibody present; HBV DNA is low or undetectable, and there is little or no histologic activity [11] [12]. The HBeAg-negative serological profile with active viral replication is the result of the emergence of precore and basal core promoter mutants, which result in either the absence of or reduced HBeAg expression and the resumption of viral replication despite the presence of anti-HBe [11], leading to higher risk of fibrosis, cirrhosis and hepatocellular carcinoma [11]. Others with chronic HBV may not be obviously categorized in these phases and it is referred to as grey or intermediate phase [12]. It has been suggested that staging can be simplified and clinical guidance improved by proposing a new three stage model of immune tolerance, immune clearance followed by immune control [13]. Though disease staging is an important step for the effective management of HBV infection, little information is available at the sub-county level.

The proper management of chronic hepatitis B infection depends on the interpretation of the HBsAg, HBsAb, HBcAb-IgM, HBeAg, and HBeAb serological markers in conjunction with the quantitative estimation of viral load [14]. The presence of HBsAg and anti-HBc are necessary to diagnose HBV infection but the presence of HBsAb, HBeAg, HBeAb and the serum HBV DNA level are useful in determining the phase of chronic infection and monitoring disease progression [15] [16]. This integrated approach allows disease staging, risk stratification, and timely initiation of antiviral therapy, resulting in better clinical outcomes, in high resource settings [12]. However, in resource-limited areas like hospital visits in North Rift region, implementing the serological and viral load test was limited due to inadequate laboratory facilities and lack of regular availability of HBV DNA testing. Therefore, the diagnosis and follow-up depend mainly on the HBsAg result. Therefore, it is critical to define HBV serological profiles and the course of viral load in patients infected with HBV to direct clinical care and optimize disease surveillance and evidence-based public health measures.

Consequently, the aim of this study was to describe the serological markers, as well as the pattern of the viral load of people with chronic HBV infection who were attending the Moi Teaching and Referral Hospital and Marigat Sub-County Hospital.

2. Materials and Methods

2.1. Study Area

The study was conducted between the years 2022 and 2024 at Moi Teaching and

Referral Hospital and Marigat Sub-County Hospital, Kenya. The hospitals are public healthcare facilities serving a semi-arid region with a high endemic burden of HBV infection.

2.2. Study Design and Population

This cross-sectional study included individuals aged 18 years and above who had a confirmed diagnosis of chronic HBV infection and were receiving clinical care at Moi Teaching and Referral Hospital and Marigat Sub-County Hospital

2.3. Sample Size Determination and Sampling Procedure

Participants came from North Rift Region selected facilities (Marigat and Moi Teaching hospitals). The calculated sample size was 85 chronic Hepatitis B patients. However, due to exclusions and incomplete data, a final sample of 65 participants was included in the analysis. The formula for sample size calculation was

$$n_0 = \frac{Z^2 \cdot p(1-p)}{e^2}, n = \frac{n_0}{1 + \frac{n_0 - 1}{N}}$$

where n = final sample size, n_0 = initial sample size; N = total population, $Z = 1.96$ (for 95% statistic level of confidence), $p = 0.5$ (maximum variability, used when the true proportion is unknown) and $e = 0.10$ (10% margin of error) was used to recruit participants in this study [17].

2.4. Eligibility Criteria

Participants were eligible for inclusion if they were HBsAg-positive, aged ≥ 18 years at the time of sampling, receiving antiviral therapy for HBV infection, and residents of Baringo County. Individuals who had incomplete outpatient records, or were unwilling to provide informed consent were excluded from the study.

2.5. Blood Sample Collection

A total of 5 ml of blood was collected from each participant into EDTA vacutainer tubes and transported to the Academic Model Providing Access to Healthcare (Ampath) Reference Laboratory (Eldoret, Kenya) for processing. Plasma was then separated and stored at -80°C until further analysis.

2.6. Detection of Serological Markers

LumiQuick Diagnostics, Inc.'s HBV-5 Panel Test, QuickProfile™ is a rapid qualitative immunoassay that detects five significant hepatitis B virus (HBV) serological markers, namely, HBsAg, Anti-HBs, HBeAg, Anti-HBe and Anti-HBc (IgM). The test has excellent diagnostic accuracy for all markers in plasma samples, with positive predictive value (PPV), negative predictive value (NPV) and overall accuracy of 99.6% for each marker. Likewise, for HBeAg, the PPV accuracy is 99.4%, NPV accuracy is 99.6%, and the test accuracy is 99.6%. Anti-HBs yields a PPV of

97.6%, NPV of 98.2%, and accuracy of 97.9%, while Anti-HBc (IgM) shows a PPV of 97.1%, NPV of 97.8%, and accuracy of 97.4%. Anti-HBe demonstrates a PPV of 96.9%, NPV of 98.8%, and overall accuracy of 98.2% [17].

2.7. HBV DNA Quantification Methods

HBV DNA was isolated from serum samples with Sansure Ultra HBV Manual Extraction Kit following the manufacturer's protocol. Briefly, serum samples were thawed at room temperature, vortexed and then centrifuged prior to extraction. Each sample was lysed for nucleic acids with proprietary lysis buffers and the nucleic acids bound to magnetic beads in the presence of an internal control to monitor nucleic acid extraction efficiency (400 µL of each sample). The bound nucleic acids were washed first with impurities and subsequently with the provided wash solutions in order to remove inhibitors, through magnetic separation. The purified nucleic acids were eluted in 30 µL of elution buffer and amplified immediately downstream. HBV DNA was quantified by real-time polymerase chain reaction (qPCR) assay with the Sansure Ultra HBV Amplification Kit according to the manufacturer's instructions. Total volume of PCR reactions was 50 µL containing HBV PCR mix, enzyme mix and 20 µL of extracted DNA. Negative controls, internal controls and HBV quantitative reference standards were included in each run to validate the assay, and ensure accurate quantification. Amplification was performed on the real-time PCR instrument under the conditions: uracil-DNA glycosylase (UDG) reaction at 50°C for 2 minutes; inactivate UDG at 94°C for 5 minutes. This was followed by 45 cycles of denaturation at 94°C for 15 seconds and annealing/extension with the acquisition of fluorescence data at 57°C for 30 seconds. Both the HBV DNA and the internal control were detected in the FAM channel, while the HEX/VIC channel was used for the internal control. HBV DNA was quantified by comparison with the quantitative reference standards provided as per the manufacturer's instructions.

2.8. Data Analysis

All statistical analyses were conducted using Stata version 18 (StataCorp, College Station, TX, USA). Categorical variables were summarized as frequencies and percentages, and group comparisons were performed using Pearson's chi-square test or Fisher's exact test, as appropriate. Fisher's exact test was applied when more than 20% of the expected viral load counts were less than 5 or when any expected count was less than 5 copies/mL. Viral load values were reported as medians with interquartile ranges (IQR). The Mann-Whitney U test was used to compare medians between two independent groups. A p-value of <0.05 was considered statistically significant.

2.9. Ethical Consideration

The study protocol was approved by the Maseno University Ethics and Review Committee (MUERC); reference number MSU/DRPI/MUERC/01121/22 and the

National Commission for Science, Technology & Innovation (NACOSTI) License No: NACOSTI/P/23/31205. The study was done following the guidelines from the Helsinki Declaration and all the participants provided written informed consent to participate in this study.

3. Results

3.1. Participant Recruitment Flow Diagram

Figure 1 shows the study participant selection and inclusion process. A total of 85 patients were recruited between January 2022 and September 2024. Of these, 20 patients were excluded from the analyses, leaving 65 patients included in the serological marker profile analysis. Among the 65 patients, 9 did not have evaluable HBV DNA results because 5 samples were not collected and 4 samples had HBV DNA quantification results that were out of range. Consequently, 56 of the 65 patients had evaluable HBV DNA measurements and were included in the HBV DNA analysis.

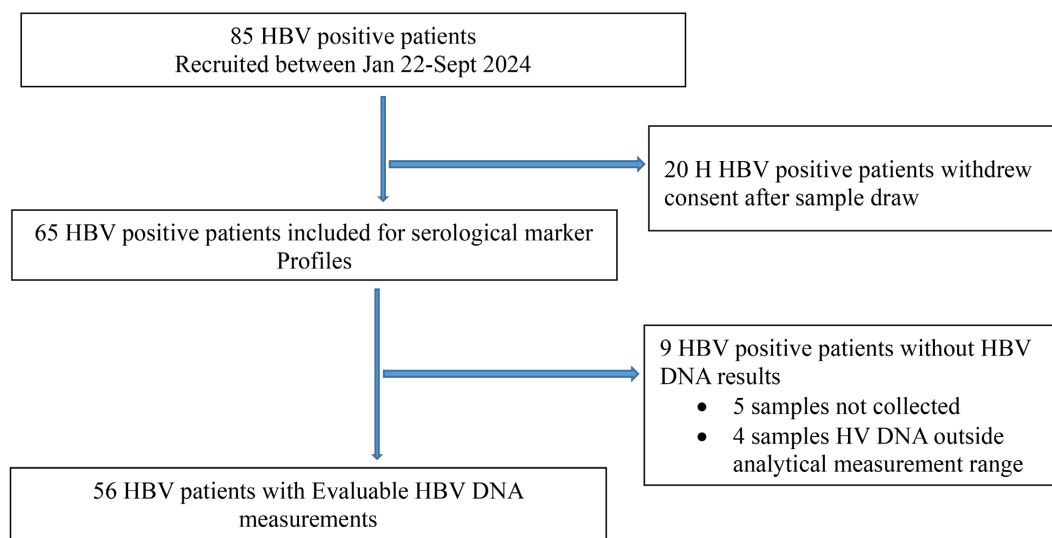


Figure 1. Participant recruitment flow diagram for 85 chronic hepatitis B patients.

3.2. Characteristics of the Study Participants

Table 1 summarizes the baseline characteristics, HBV serological markers, and viral load distribution among the study participants. A total of 65 HBsAg-positive individuals were included in the study, with ages ranging from 20 to 72 years and a mean age of 39.5 years. The largest proportion of participants (31, 47.7%) were aged above 40 years, while 11 (16.9%), 13 (20.0%), and 10 (15.4%) participants were aged 18 - 25, 26 - 33, and 34 - 40 years, respectively. The distribution of participants across the age categories differed significantly ($\chi^2 (3) = 36.875$, $p < 0.001$). Male participants constituted 38 (58.5%) of the study population, while 27 (41.5%) were female. However, the difference in the proportion of males and females was not statistically significant, ($\chi^2 (1) = 2.250$, $p = 0.134$).

Table 1. Baseline demographic characteristics, HBV serological markers, and HBV DNA levels among study participants.

Variable	Category	N (%)	HBsAg f(%)	HBsAb f(%)	HBeAg f(%)	HBeAb f(%)	HBcAb-IgM f(%)	Median HBV DNA (IU/mL) (P25 - P75)	p-value
Gender	Female	27 (41.5)	27 (100)	2 (7.4)	0 (0)	3 (11.1)	7 (25.9)	68 (0.54 - 644)	p = 0.088 (Mann-Whitney U)
	Male	38 (58.5)	38 (100)	3 (7.9)	0 (0)	11 (28.9)	8 (21.1)	187 (12.4 - 216,072)	
Age	18 - 25	11 (16.9)	11 (100)	1 (9.1)	0 (0)	2 (18.2)	2 (18.2)	96 (0.1 - 766,174)	p = 0.529 (Kruskal-Wallis test)
	26 - 33	13 (20.0)	13 (100)	2 (15.4)	0 (0)	1 (7.7)	3 (23.1)	2440 (32 - 1,596,525,843)	
	34 - 40	10 (15.4)	10 (100)	0 (0)	0 (0)	2 (20.0)	2 (20.0)	126 (0.18 - 4,767,331)	
	>40	31 (47.7)	31 (100)	2 (6.5)	0 (0)	9 (29.0)	8 (25.8)	108 (6.6 - 48,514)	
Treatment	Entecavir	22 (33.8)	22 (100)	2 (9.1)	0 (0)	1 (4.5)	6 (27.3)	28.3 (2.3 - 2740)	p = 0.335 (Mann-Whitney U)
	TDF/FTC	43 (66.2)	43 (100)	3 (7.0)	0 (0)	13 (30.2)	9 (20.9)	187 (6.3 - 109,667)	

All participants tested positive for hepatitis B surface antigen (HBsAg) (65, 100%). Hepatitis B surface antibody (HBsAb) was detected in 5 participants (7.7%), whereas none of the participants tested positive for hepatitis B e antigen (HBeAg). Hepatitis B e antibody (HBeAb) was identified in 14 participants (21.5%), while hepatitis B core antibody IgM (HBcAb-IgM) was detected in 15 participants (23.1%).

The median HBV DNA viral load was higher among males at 187 IU/mL (IQR: 12.4 - 216,072) compared to females, who had a median viral load of 68 IU/mL (IQR: 0.54 - 644). However, this difference was not statistically significant (Mann-Whitney U test, $p = 0.088$).

Participants aged 26 - 33 years demonstrated the highest median HBV DNA viral load of 2440 IU/mL (IQR: 32 - 1,596,525,843), whereas those aged 18 - 25 years had a median viral load of 96 IU/mL (IQR: 0.1 - 766,174), participants aged 34 - 40 years had 126 IU/mL (IQR: 0.18 - 4,767,331), and those aged above 40 years had 108 IU/mL (IQR: 6.6 - 48,514). No statistically significant difference in HBV DNA levels was observed across age categories (Kruskal-Wallis test, $p = 0.529$).

The antiviral treatment regimen for 22 participants (33.8%) was Entecavir, while 43 (66.2%) were on tenofovir disoproxil fumarate/emtricitabine (TDF/FTC). Participants receiving TDF/FTC had a higher median HBV DNA viral load of 187 IU/mL (IQR: 6.3 - 109,667) compared to those on Entecavir, who had a median viral load of 28.3 IU/mL (IQR: 2.3 - 2740). However, the difference in viral load between treatment groups was not statistically significant (Mann-Whitney U test, $p = 0.335$).

3.3. Serological Marker Profiles and Corresponding HBV DNA Viral Load Levels

Table 2 summarizes the distribution of HBV serological marker profiles and the corresponding HBV DNA viral load levels among study participants. The most common serological marker profile characterized by HBsAg positivity with negative HBsAb, HBeAg, HBeAb, and HBcAb-IgM, was observed in 31 participants (47.7%). This group had the lowest median HBV DNA viral load of 59 IU/mL (IQR: 0.01 - 1899) and is consistent with HBeAg-negative chronic HBV infection. Participants who were HBsAg positive with detectable HBcAb-IgM but negative HBeAg and HBeAb accounted for 15 (23.1%) of the study population. This profile is suggestive of either early acute infection or a possible reactivation phase. This group demonstrated a higher median viral load of 187.0 IU/mL (IQR: 6.44 - 1530346.00), indicating greater variability in viral replication activity among individuals within this category. Fourteen participants (21.5%) exhibited a profile characterized by HBsAg and HBeAb positivity, but negative HBsAb, HBeAg, and HBcAb-IgM, consistent with HBeAg-negative chronic HBV infection following HBeAg seroconversion. This group had a median HBV DNA viral load of 115.43 IU/mL (IQR: 2.80 - 64338680.00), indicating substantial heterogeneity in viral replication despite HBeAg negativity. A minority of participants (5, 7.7%) demonstrated concurrent HBsAg and HBsAb positivity, representing a serologically discordant or atypical profile. This group exhibited the highest median HBV DNA viral load of 2440.0 IU/mL (IQR: 181.30 - 3055054.00), suggesting ongoing viral replication despite detectable surface antibody. Overall DNA viral load levels varied considerably across the different serological marker profiles. However, the differences in median viral load among the four groups were not statistically significant (Kruskal-Wallis $H = 2.440$, $df = 3$, $p = 0.486$).

The overall frequency of HBsAb positivity among the 56 patients with evaluable HBV DNA level was 5 (8.9%) and the majority were HBsAb negative (51, 91.1%). Patients who were HBsAb positive had a higher median viral load of 1642 copies/mL (IQR: 567 - 11,466) compared to those who were HBsAb negative, who had a lower median viral load of 404 copies/mL (IQR: 29 - 50,000). For HBeAb, 12 patients (21.4%) were positive, while 44 (78.6%) were negative. Interestingly, the median viral load for HBeAb-positive patients was found to be 17,589 copies/mL (IQR: 118 - 27,400,000) while that for HBeAb-negative patients was 457 copies/mL (IQR: 30 - 35,000), indicating that even in a subset of seroconverters or HBeAg-negative chronic infection cases, viral replication is significant. In the case of HBcAb-IgM, 14 patients (25.0%) were positive while 42 (75.0%) were negative. There was a difference in the median viral load between the HBcAb-IgM-positive and HBcAb-IgM-negative groups: 162 copies/mL (IQR: 28 - 1,500,000) for the former group and 723 copies/mL (IQR: 64 - 33,333) for the latter group, suggesting some heterogeneity in viral replication in acute and chronic presentations. All 56 patients (100%) were HBsAg reactive and HBeAg negative, which meant that only infected patients with resolved or omitted expression of e-antigen were in-

cluded. Despite uniform HBsAg positivity and HBeAg negativity, the overall median viral load of the cohort was 532 copies/mL (IQR: 40 - 50,000) with wide inter-individual variation in HBV replication (**Table 3**).

Table 2. Serological marker profiles and corresponding HBV DNA viral load levels.

Serological profile	Interpretation	N (%)	Median HBV DNA (IU/mL) (IQR: 25 th - 75 th Percentile)	p-value
HBsAg (+), HBsAb (-), HBeAg (-), HBeAb (-), HBcAb-IgM (-)	Chronic HBV infection (inactive infection or low-replication phase)	31 (47.7%)	59 (0.01 - 1899)	p = 0.486 (Kruskal-Wallis test)
HBsAg (+), HBsAb (-), HBeAg (-), HBeAb (-), HBcAb-IgM (+)	Acute HBV infection or chronic HBV reactivation phase	15 (23.1%)	187 (6.44 - 1,530,346)	
HBsAg (+), HBsAb (-), HBeAg (-), HBeAb (+), HBcAb-IgM (-)	HBeAg-negative chronic Hepatitis B infection	14 (21.5%)	115.43 (2.80 - 64,338,680)	
HBsAg (+), HBsAb (+), HBeAg (-), HBeAb (-), HBcAb-IgM (-)	Serologically discordant or atypical profile	5 (7.7%)	2440 (181.3 - 3,055,054)	
Total	-	65 (100%)		

Table 3. Viral load distributions across serological marker categories (n = 56).

Serological Marker	Category	N (%)	Median Viral Load (CP/mL) IQR25 - IQR75
HBsAb	Positive	5 (8.9)	1642 (567 - 11,466)
	Negative	51 (91.1)	404 (29 - 50,000)
HBeAb	Positive	12 (21.4)	17,589 (118 - 27,400,000)
	Negative	44 (78.6)	457 (30 - 35,000)
HBcAb-IgM	Positive	14 (25.0)	162 (28 - 1,500,000)
	Negative	42 (75.0)	723 (64 - 33,333)
HBsAg	Reactive	56 (100)	532 (40 - 50,000)
HBeAg	Negative	56 (100)	532 (40 - 50,000)

Figure 2 illustrates that patients receiving Entecavir exhibited a lower median HBV DNA level (255 copies/mL) compared to those on TDF/FTC therapy (1075 copies/mL). This difference may reflect variations in disease severity, treatment response, or levels of adherence among the patient groups.

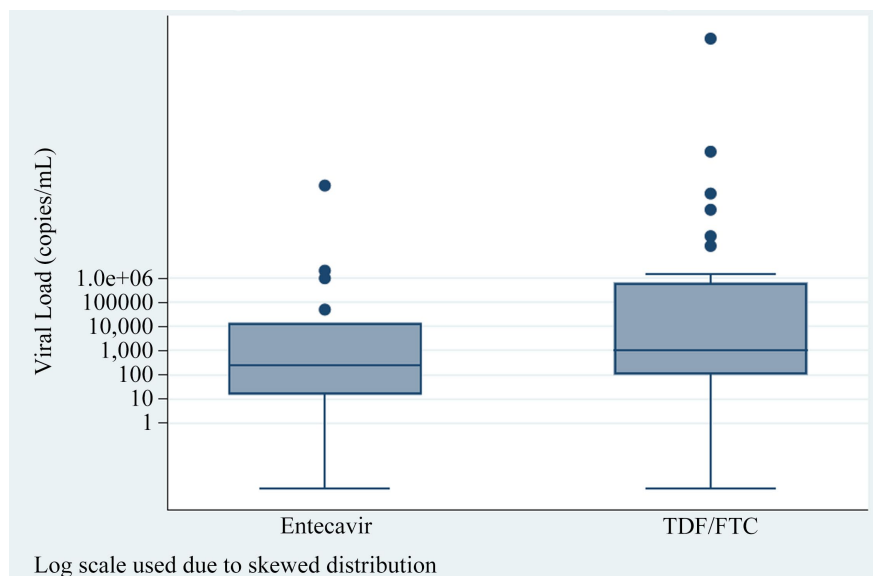


Figure 2. Viral load distribution by treatment regimen.

4. Discussion of Results

Sixty-five chronic hepatitis B virus (HBV) infected individuals who presented for care in the endemic areas of Kenya had their serological profile and viral load distribution analyzed and categorized as shown in **Table 1**. There was an 100% positivity rate for all participants in the testing procedure, indicating chronic infection. Prior studies have shown that protective HBsAb seroconversion and subsequent production of HBsAb occur less frequently in the long-term setting of NA therapy (often <10% during extended therapy) [18]; baseline immune competence, including the presence of HBsAb-specific B cells, has been linked to higher rates of HBsAb seroconversion, implying that host factors might constrain the ability to produce protective antibodies in most NA-treated patients. The prevalence of hepatitis B surface antibody (HBsAb) was found to be similar in male and female subjects (7.9% vs 7.4%). The prevalence of HBeAb was higher among males (28.9%) than among females (11.1%) though the difference was not statistically significant ($p = 0.085$). The findings from published literature generally agree that sex differences in HBV infection and immune responses exist. A number of studies have indicated that males are at increased risk of chronic HBV infection and are more seropositive for some HBV markers or viral persistence than females. For instance, males were found to be more chronically HBV-positive and infected than females, perhaps due to immunological and hormonal differences between males and females, such as greater innate and adaptive immune responses in fe-

males [19]. Sero-epidemiological studies in various regions revealed that females were generally better controllers of HBV infection than males, and that HBV infection outcomes differed by individual serological markers (e.g., HBeAb) and region-specific setting and genotype [19]. In other studies, there were documented sex differences in the prevalence of HBeAg and in disease progression and some studies indicated a higher prevalence of HBeAg in male cohorts, but not all differences were significant. For instance, in a cross-sectional study, HBeAg positivity was greater in male than female patients but this did not reach significance when the data were analyzed [20].

HBeAg is frequently associated with a transition to the inactive carrier state and male patients may be more likely to achieve partial immune control of HBV. Interestingly, males had significantly higher median viral loads, 6554 CP/mL, than females, 171 CP/mL, indicating either more viral replication or a delayed response to treatment. This is consistent with current evidence of sex differences in the dynamics of HBV and disease progression. While some studies do not show any significant differences, a systematic multinational study revealed that males had higher levels of detectable HBV DNA at baseline than females, which suggested higher levels of HBV viral persistence in some studies, even with antiviral therapy [21].

No statistically significant difference was seen in prevalence of HBcAb-IgM (7.7% of females were positive compared to 7.0% of males, $p = 0.646$). An age-stratified analysis revealed that those >40 years of age had the highest prevalence of HBeAb (29.0%) and HBcAb-IgM (25.8%) which suggests a higher risk for viral reactivation or reconstitution of immune system in older patients. Younger age groups showed more irregular distribution, and there were no significant differences in serological markers between the age groups. By treatment regimen, there was a significant difference in HBeAb positivity between those taking tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) (30.2%) and those taking entecavir (4.5%) ($p = 0.017$). This could represent a higher number of immune responses or earlier progression to the Low-replicative serological profile in patients receiving TDF/FTC. The median viral loads were significantly different between patients receiving entecavir (255 CP/mL) and those receiving TDF/FTC (1075 CP/mL), however, indicating better virological suppression in patients receiving entecavir (p -value = 0.017). The data, taken together, suggest that there is heterogeneous serological and virological response among treated HBV patients. TDF/FTC may induce immunologic changes in favor, but there is a higher level of viral suppression achieved with entecavir. The differences show the need to treat each patient as a unique patient by using individual treatment strategies based on both serologic and virologic parameters.

The low level of protective HBsAb in all groups also reinforces the need for improved surveillance and perhaps immune therapeutic interventions with adjuvants to bring about long-term control or functional cure. Published meta-analyses found that tenofovir had a higher proportion of patients with complete viro-

logical response and a greater decrease in HBV DNA compared to entecavir, especially in HBeAg-positive patients in terms of antiviral treatment responses. A recent systematic review concluded that tenofovir had a significantly higher probability of achieving complete virological response and greater viral load reduction compared with continued entecavir therapy [22]. A second meta-analysis also showed greater viral suppression early on with tenofovir than with entecavir at specific timepoints [23]. The serologic differences, however, between tenofovir and entecavir groups, such as HBeAg seroconversion and HBsAg reduction, tend to be less significant or not significant in these meta-analyses, particularly in longer follow-up periods [18]. This could contribute to the different correlations between treatment regimen and HBeAb seen in this study. As for age differences, the old literature generally shows that older age is linked to a more complex immunological profile of chronic HBV with slower serological transition and variable HBV viral replication, but there are no consistent reports on differences in the prevalence of HBeAb and HBcAb-IgM by age. Overall, the trends of this cohort higher median viral loads in males, variability in response to treatment between tenofovir and entecavir, low prevalence of HBsAb are similar to those in recent clinical and systematic studies. This study highlights the multi-factorial aspects of serological and virological response in chronic hepatitis B and the need to take host and treatment factors into account when assessing these responses.

In **Table 2**, these HBV infected patients are divided into four predominant serological patterns. These patterns may be indicative of various stages of viral replication and immune response, but further clinical and longitudinal information is needed to confirm classification into clinical phases. The serological features of this population are generally similar to those that have been reported in natural history studies and clinical guidelines for chronic hepatitis B virus (HBV) infection. The most common profile in this study was the absence of the other markers in association with the presence of HBsAg (47.7%), and this profile is compatible with a low-replicative serological profile of chronic HBV infection. Profiles of persistent HBsAg with low or variable viral replication are known in the literature, and represent chronic infection with little immune response, and are usually low disease activity, unless reactivated. In clinical practice, chronic HBV natural history is characterized by immune-tolerant, immune-active, inactive carrier and reactivation stages, which are marked by appropriate serological and virological factors [24]. Besides the usual serologic phases, another large proportion (23.1% in the present study) had concomitant HBcAb-IgM, which is what has been observed in other groups of patients with serologic phases of immune-mediated viral clearance or reactivation. The immune-activating phase, characterized by the host's cytotoxic response and possible hepatic inflammation, is well characterized in reviews of the natural history of HBV and has been correlated with the changes in serological markers and virological activity [16]. The serological pattern of HBeAb seropositivity without IgM (in 21.5% of patients) is similar to the HBeAg seroconversion pattern seen in natural history cohorts in which the emergence of anti-

HBe indicates a shift from high replicative states to immune control. The same percentage of HBeAg-negative, anti-HBe positive individuals have been reported in untreated chronic HBV populations and it has been stressed that this phase can encompass two groups: inactive carriers and HBeAg-negative chronic hepatitis B, especially in the presence of core promoter or precore variants [25]. Low prevalence of HBsAb positivity (7.7%) also confirmed that anti-HBs antibody is not a common finding in seroprevalence studies of chronically infected people not undergoing functional cure or seroconversion, indicating that the lack of this antibody is a typical feature of chronic infection without seroconversion.

A large seroepidemiologic study has reported that the presence of HBsAb in HBsAg-positive individuals is associated with low levels of HBsAg and does not necessarily reflect the end of viral replication [26]. Rare and atypical serologic patterns, such as simultaneous positivity for HBsAg and HBsAb, have been found in other African cohorts, although the prevalence has ranged but these patterns have led to the conclusion that discordant serology is not unique to this study. Such unusual profiles could be due to immune escape variants, assay restrictions, or complicated interactions between host and virus [26]. The serological distribution of this cohort is consistent with the diversity of phases of chronic hepatitis B natural history observed worldwide. The relative high percentage of patterns that were suggestive of immune activity or transition in this treated population indicates the need for integrated serological and virological monitoring as recommended in guidelines and epidemiological studies to guide treatment decisions and to predict disease progression [24].

Figure 2 shows the distribution of the levels of HBV viral load in patients on two different antiviral treatment regimens. HBV DNA levels were also lower in patients receiving entecavir in this group (255 copies/mL; IQR, 15 - 13,932) than in patients receiving TDF/FTC (1075 copies/mL; IQR, 103 - 651,000). This difference was not statistically compared in the present analysis, but may indicate early viral suppression that is more effective for this population in the entecavir group. This finding should be used with caution, however, because clinical and virological differences at baseline were not controlled for between treatment groups. So far, the literature has presented conflicting but more or less similar results comparing the efficacy of entecavir with tenofovir-based treatments in chronic HBV treatment. Several systematic reviews and meta-analyses have shown that, in nucleos(t)ide analogue-naïve patients, both agents are equally effective at achieving HBV DNA suppression, ALT normalization, and HBeAg seroconversion [22]. Likewise, both drugs were shown to be equally effective in the long-term, and viral suppression in HBeAg serological outcomes was comparable, with tenofovir appearing to achieve viral suppression earlier than cTPO in some studies [24].

A large multicentre retrospective study showed comparable long-term efficacy of both agents, with entecavir having a greater effect in reducing HBV DNA levels in the first month of treatment, 6 and 12 months, while tenofovir had a higher probability of complete viral suppression during the entire treatment period (haz-

ard ratio = 1.66; 95% CI 1.21 - 2.33; $p = 0.010$) [27]. Another systematic review and meta-analysis also found that tenofovir might be more effective than entecavir for long-term viral suppression, especially in nucleos(t)ide-naïve patients, based on a higher overall rate of HBV DNA suppression, though some time-specific differences (such as 24 or 48 weeks) were not consistently different across studies [18]. Further, high baseline HBV DNA levels among treatment naïve patients have demonstrated more patients had undetectable HBV DNA at the end of defined treatment periods with TDF than with entecavir [22].

However, some studies have found that TDF and entecavir are similarly effective in suppressing HBV DNA over the long term, with no statistically significant difference in HBV DNA suppression, normalization of alanine aminotransferase and seroconversion rates at 24 and 48 weeks of therapy [18]. These results suggest that although both agents are effective first-line drugs recommended by the World Health Organization, the effects they have on patient subgroups may differ, as may their efficacy be based on initial viral load and the length of treatment. The greater width and higher upper quartiles of viral load seen in the TDF/FTC group in this study could be due to patient-specific factors, including differences in viral genetic factors, adherence to the regimen, or disease severity at treatment entry. Considering this evidence collectively, it appears that entecavir and tenofovir are both highly effective for suppressing HBV DNA, and some studies suggest that there may be an advantage for tenofovir in suppressing HBV-DNA completely in certain subpopulations, whereas other studies suggest that the long-term efficacy of the two regimens is comparable.

In this study, alanine aminotransferase (ALT) levels or longitudinal follow-up data were not accessed; further, the study did not classify these into established clinical phases of chronic HBV infection. Moreover, the lack of treatment duration, adherence statistics, and the baseline disease severity do not provide the possibility to assess a response to treatment and disease progression comprehensively.

5. Conclusion

This study demonstrated diverse serological and viral load patterns among patients with chronic hepatitis B infection in a hyperendemic setting. Patients who do not achieve adequate viral suppression, particularly those on TDF/FTC regimens, should be further evaluated for possible drug resistance, suboptimal adherence, or the need for treatment modification. HBV DNA levels and HBeAg status are not directly correlated, as all samples with detectable viral load were HBeAg non-reactive. This indicates that, among individuals with chronic hepatitis B, persistent viral replication can occur independently of HBeAg expression. The findings also highlight variability in the severity of chronic hepatitis B infection and differences in treatment response. While most patients exhibited low-replicative serological patterns, some showed evidence of ongoing immune activity or immune recovery. These patterns provide important insights for both clinical decision-making and public health interventions.

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Disclaimer

This study was conducted for academic and research purposes only. The information, data, and findings presented herein are based on the sources, methods, and participants involved in the study. While every effort has been made to ensure accuracy, reliability, and validity, the authors do not make any guarantees regarding completeness or applicability of the results beyond the scope of the research. The views and interpretations expressed in this work are solely those of the author(s) and do not necessarily represent the official positions of any institution, organization, or funding body. This research is not intended to provide medical, legal, financial, or professional advice. Readers are encouraged to exercise their own judgment and seek professional guidance where appropriate. Participation in the study was voluntary, and confidentiality and ethical standards were observed throughout the research process.

Conflicts of Interest

The authors declare the following competing interests: Dr Missiani Ochwotto, and Dr Lilian Ogonda supported putting the intellectual content together, alongside Dr Carolyne Kifude and served as advisors during the conduct of the exercise. All other authors declare no competing interests.

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