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Detection of HBeAg versus Viral DNA for the Management of People Infected with the Hepatitis B Virus in Burkina Faso, West Africa

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Abstract

HBeAg serves as a marker of wild-type viral replication of the hepatitis B virus (HBV). The detection of HBeAg is of significant clinical importance in the classification of chronic hepatitis B. Given the high frequency of mutations that can occur in the HBeAg gene, viral DNA detection is the most accurate marker of viral replication. In countries with limited resources, the cost of molecular tests represents a significant obstacle to their accessibility. Accordingly, the World Health Organization (WHO) recommends the use of HBeAg in conjunction with alanine aminotransferase (ALT) for the purpose of making therapeutic decisions. The objective of this study was twofold: firstly, to evaluate the suitability of HBeAg and HBV DNA for the clinical management of individuals infected with HBV in Bobo-Dioulasso, Burkina Faso; secondly, to ascertain the diagnostic performance of the Enzyme-Linked Fluorescent Assay (ELFA) for the detection of HBeAg. This cross-sectional study was conducted from March 1, 2023, to February 29, 2024, at the "Assaut-Hépatites" center in Bobo-Dioulasso. The study population consisted of patients who were HBsAg-positive and who presented to the laboratory for HBeAg testing and DNA quantification as part of an initial evaluation. HBeAg testing was conducted using the VIDAS HBe/Anti-HBe kit (Biomérieux, Marcy-l'Etoile, France),

while HBV DNA quantification was performed by real-time PCR using the GENERIC HBV VIRAL LOAD version 2.0 (GHBV-CV) kit (BIOCENTRIC, Bandol, France). A total of 128 patients who tested negative for hepatitis B surface antigen (HBsAg) were included in the study. The mean age of the participants was 34.82 ± 11.02 years. The presence of HBeAg was identified in 5.5% (7/128) of participants. The presence of HBV DNA was confirmed in all participants, and 7.8% (10/128) exhibited a viral load (VL) exceeding 20,000 IU/mL. The sensitivity of the VIDAS HBe/anti-HBe test ranged from 18.51% for a viral DNA detection threshold of >2000 IU/mL to 75.00% for a threshold of >2,000,000 IU/mL. Notwithstanding the low diagnostic sensitivity of the test, the results demonstrated that there was no statistically significant difference between the proportions of individuals eligible for treatment on the basis of HBeAg (5.5%) and VL (7.8%).

Keywords

Hepatitis B, HBeAg, HBV DNA, Adequacy, Clinical Management, Diagnostic Performance, Burkina Faso

1. Introduction

Chronic hepatitis B represents a significant global public health concern, as evidenced by data from the World Health Organization [1]. As reported by the World Health Organization, 254 million individuals were living with chronic hepatitis B in 2022 worldwide, with approximately 1.1 million deaths attributed to cirrhosis or hepatocellular carcinoma [2]. In Africa, 65 million individuals are chronically infected with the hepatitis B virus [2]. Chronic hepatitis B is the primary cause of hepatocellular carcinoma (HCC) and liver cirrhosis in sub-Saharan Africa [3] [4]. The management of hepatitis B virus infection requires the monitoring of a range of virological markers, including HBsAg, HBeAg, HBeAc, and HBV-DNA.

HBV-DNA serves as an accurate marker of replication, and its quantification is crucial in the context of therapeutic decision-making. Nevertheless, in countries with constrained resources, the lack of access to molecular biology tools represents a significant obstacle, largely due to the inadequacy of the available platforms and the financial burden associated with conducting the examination. In such countries, the WHO recommends the use of HBeAg, which is a marker of wild virus replication, in conjunction with alanine aminotransferase (ALT) for the purpose of making therapeutic decisions [4] [5]. HBeAg is synthesized by the C gene, and mutations in this gene can affect HBeAg synthesis without affecting viral replication [6]-[8]. Consequently, HBeAg may be undetectable in serum despite ongoing viral replication. Gene C mutants represent a significant public health concern, frequently leading to misdiagnosis and an accelerated progression to liver complications such as cirrhosis and hepatocellular carcinoma (HCC) [7] [8].

Hepatitis B is a significant public health concern in Burkina Faso, with an estimated 9% of the general population infected [9] [10]. Of these cases, 2 million are chronic carriers [9]. In clinical practice in Burkina Faso, 90% of individuals with chronic hepatitis B virus (HBV) infection are HBeAg-negative. As in developing countries, quantification of HBV-DNA represents a significant challenge. Consequently, HBeAg is widely employed to assess viral replication, thereby facilitating the formulation of an appropriate therapeutic plan.

The availability rapid diagnostic tests (RDTs) for HBeAg represents a promising solution, particularly in resource-limited countries where RDTs are widely preferred due to their ease of use and affordability. However, despite these advantages, studies have revealed that some HBeAg-RDTs exhibit unsatisfactory analytical performance [4] [11] [12]. To achieve optimal results in the detection of HBeAg, it is recommended to utilize more efficient immunological tests, such as enzyme immunoassay (EIA) [4].

The VIDAS HBe/Anti-HBe is an automated qualitative test for the detection of HBeAg or antibodies (anti-HBe) by the Enzyme-Linked Fluorescent Assay (ELFA) technique. It is incorporated into the system for the surveillance of individuals infected with virus HBV in Burkina Faso. Despite the extensive utilization of this test, there is a dearth of data concerning its diagnostic performance in comparison with real-time PCR for HBV DNA quantification. Consequently, the present study was designed to assess the suitability of HBeAg and viral DNA for the management of HBV-infected individuals at the "Assaut-Hépatites" Center in Bobo-Dioulasso, Burkina Faso, and also to determine the diagnostic performance of the ELFA technique for HBeAg detection.

2. Materials and Methods

2.1. Study Design - Population - Setting

This cross-sectional study was conducted over a period of 12 months, from March 2023 to February 2024. The study population consisted of patients who tested positive for the hepatitis B surface antigen (HBsAg) and presented to the biomedical analysis laboratory of the "Assaut-Hépatites" center in Bobo-Dioulasso during the study period for testing of the hepatitis B e antigen (HBeAg) and the hepatitis B virus (HBV) deoxyribonucleic acid (DNA) as part of an initial check-up. The "Assaut-Hépatites" center is a specialized facility that provides care for individuals who are chronically infected with viral hepatitis B and C. It is situated in Bobo-Dioulasso, the second-largest city in Burkina Faso. The center is structured into three departments: the Clinical Department, which oversees the clinical follow-up of patients; the Laboratories Department, where biological analyses are conducted; and the Data Management Department.

2.2. Data and Sample Collection

The data were collected via a structured questionnaire based on the information presented in the analysis bulletin. In the event of incomplete or absent data, the

information was obtained directly from the patient. Data collection was conducted by laboratory technicians who had received training in research ethics and data collection. The data collected included sociodemographic and clinical information. Approximately 8 mL of whole blood was collected from each consenting patient via venipuncture. The blood samples were labeled with the date of collection and the patient ID number and then processed. The serum obtained was aliquoted into two cryotubes, which were clearly labeled with the patient ID number and the date of collection. One cryotube was used for HBeAg and HBV DNA viral load testing, while the second was stored at −80˚C for future studies.

2.3. Biological Analysis

Detection of HBeAg

The presence of HBeAg was determined through the utilization of the VIDAS HBe/Anti-HBe kit (Biomérieux, Marcy-l'Etoile, France) on the mini VIDAS® instrument. The VIDAS HBe/Anti-HBe Kit is an automated qualitative test that employs the enzyme-linked fluorescence assay (ELFA) as its detection method. The HBe test is based on the combination of enzyme-linked immunoassay and final fluorescence detection. Upon completion of the process, the test index is calculated, thereby enabling the interpretation of the analysis. A negative result is indicated by an index value of less than 0.1, while a positive result is indicated by an index value of 0.1 or greater. The analytical sensitivity of the HBeAg test, determined using the Paul-Ehrlich-Institut (PEI) standard provided by the manufacturer, was 0.25 PEI U/mL, and its specificity was greater than 98%.

Quantification of HBV DNA

Quantification of hepatitis B virus (HBV) DNA was conducted through real-time polymerase chain reaction (PCR). The DNA extraction was conducted using the GenoXtract® instrument with GXT NA extraction kits (Hain Lifescience, Nehren, Germany). An initial serum volume of 500 µL was used for an elution volume of 50 µL. HBV DNA amplification was conducted using the generic HBV viral load assay (GHBV-CV) (BIOCENTRIC, Bandol, France) on the FluoroCycler® XT (Hain Lifescience, Nerhen, Germany). The assay has a detection limit of 5 IU/mL.

2.4. Statistical Analyses

The statistical analyses were conducted using R software, version 4.3.0. The proportions of biological parameters were calculated. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (CI) were calculated by comparing the ELFA results with those of the different viral DNA levels. The significance level for the analyses was set at $p \leq 0.05$.

2.5. Ethical Considerations

This study did not require ethical committee approval. All biological analyses

were performed as part of routine procedures, and no additional examinations were conducted beyond those that the patient had undergone prior to coming to the laboratory. Nevertheless, all participants were informed, and informed consent was obtained. Furthermore, data generated during this study were identified with a unique identification code to ensure anonymity and confidentiality. The information was stored in a protected database accessible only to the investigators.

3. Results

3.1. Socio-Demographic Characteristics of the Participants

A total of 128 patients were included in the study. The mean age of the participants was 34.82 ± 11.02 years. The male population constituted 57.0% (73/128), resulting in a sex ratio of 1.32. The age group with the highest representation was 25-35, comprising 50 patients (39.06%). Regarding occupation, over half (54.7%) of the population were self-employed. Table 1 illustrates the distribution of participants by socio-demographic characteristics.

Table 1. Distribution of participants by socio-demographic characteristics.

Characteristic	N = 128	Percentage (%)
Sex		
Male	73	57.0
Female	55	43.0
Age group (year)		
16 - 25	30	23.4
26 - 35	50	39.1
36 - 45	27	21.1
+45	21	16.4
Occupation		
Student	17	13.3
Employee	41	32.0
Informal sector workers	70	54.7

3.2. Detection of HBeAg and Quantification of HBV DNA

Of the 128 individuals included in the study, HBeAg was detected in seven (5.5%) patients. Regarding HBV-DNA, it was detectable in all (100%) patients, and 10 (7.8%) exhibited a viral load (VL) greater than 20,000 IU/mL (Table 2).

3.3. Performance of the ELFA Technique in Comparison with Real-Time PCR for the Detection of Viral Replication

The diagnostic performance of the ELFA, VIDAS HBe/anti-HBe technique was evaluated according to varying thresholds of viral DNA quantity. The sensitivity

of the ELFA, VIDAS HBe/anti-HBe technique was observed to increase with rising levels of viral DNA detected, with figures ranging from 18.51% to 75% for viral DNA amounts of 3.30 log10 IU/mL and 6.30 log10 IU/mL, respectively. As the amount of viral DNA detected increased, the specificity of the ELFA technique exhibited a corresponding decline. Table 3 illustrates the performance of the ELFA technique as a function of varying viral load thresholds.

Table 2. Detection of HBeAg and of HBV DNA quantification.

	N = 128	Proportions (CI 95%)
AgHBe		
Negative	121	94.5% (89.0 - 97.8)
Positive	7	5.5% (2.2 - 10.9)
HBV DNA		
VL < 20,000 IU/ml	118	92.2% (86.1 - 96.2)
VL ≥ 20,000 IU/mL	10	7.8% (3.8 - 13.9)

Table 3. Performance of the ELFA technique according to viral load thresholds.

Viral load positivity threshold	Viral burden	ELFA AgHBe		Se (CI 95%)	Sp (CI 95%)	PPV (CI 95%)	VPN (CI 95%)
		Positive	Negative				
>3.30 log10 IU/mL 2000 IU/ml	Detectable	5	22	18.51 (6.30 - 38.08)	98.01 (93.02 - 99.75)	71.42 (29.04 - 96.33)	81.81 (73.77 - 88.24)
	Undetectable	2	99				
>4.30 log10 IU/mL 20,000 IU/mL	Detectable	4	6	36.36 (10.92- 69.20)	97.43 (92.42 - 99.46)	57.14 (18.40 - 90.10)	94.21 (88.44 - 97.64)
	Undetectable	3	114				
>5.30 log10 IU/mL 200,000 IU/mL	Detectable	3	3	50.0 (11.81 - 88.18)	96.72 (91.81 - 99.09)	42.85 (49.89 - 81.59)	97.52 (92.92 - 99.48)
	Undetectable	4	118				
>6.30 log10 IU/mL 2,000,000 IU/mL	Detectable	3	1	75.00 (19.41 - 99.36)	96.77 (91.94 - 99.11)	42.85 (9.89 - 81.59)	99.17 (95.48 - 99.97)
	Undetectable	4	120				

4. Discussion

The proportion of patients with HBeAg negativity was 94.5% (121/128). This result is consistent with the findings of the Ministry of Health of Burkina Faso, which estimated that over 90% of individuals were negative for HBeAg. The absence of HBeAg is believed to be indicative of a lack of viral replication for a wild-type strain of HBV [7] [8]. Therefore, our HBeAg results indicate that most individuals chronically infected with HBV are unlikely to experience viral replication. Consequently, based on the HBeAg results, only 5.5% of participants may potentially benefit from antiviral treatment.

The viral load was quantifiable in all the samples. In accordance with the World Health Organization's guidelines for viral load-based treatment criteria, 7.8% of participants (viral load exceeding 20,000 IU/mL) are eligible for antiviral treatment.

Furthermore, 2% of participants who would benefit from treatment will not be treated based on HBeAg status. This discrepancy may be attributed to the sensitivity of the ELFA technique, which employs the VIDAS HBe/anti-HBe kit for HBeAg detection. However, the discrepancy between the proportion of individuals who should be initiated on HBeAg treatment, and the viral load was not statistically significant in our findings. Additionally, in individuals with a viral load exceeding 20,000 IU/mL (2%), we did not assess alanine aminotransferase to more accurately categorize them in accordance with the WHO recommendations.

The sensitivity of the ELFA technique in conjunction with the VIDAS HBe/anti-HBe kit for the detection of HBeAg in comparison to viral load exhibited a range of 18.51% to 75.00% for DNA quantities exceeding 2000 IU/mL and 2,000,000 IU/mL, respectively. Conversely, as the viral load increased, the specificity decreased, ranging from 98.01% for a quantity of DNA greater than 2000 IU/mL to 96.77% for 2,000,000 IU/mL. Several studies have reported findings that are like those presented here. These studies demonstrate relatively low sensitivities but high specificities for DNA amounts greater than 200,000 IU/mL. For example, a study conducted in Ethiopia found a sensitivity of 50% and a specificity of 93% with the EIA (Vidas, Biomerieux) technique [13]. Similarly, Godbole *et al.* (2013) reported sensitivities of 77% and specificities of 98% for the CLIA technique (Architect Abbot and Architect Elecsys) in London [14]. These results underscore the sensitivity limitations of enzyme-linked immunosorbent assays (ELISAs) for HBeAg detection, indicating a pervasive challenge. This observation may be attributed to the absence of international standards for HBeAg determination, which could potentially impact the analytical performance of immunological assays. It was observed that the VIDAS reagent was calibrated with serum library sera. The challenge of establishing an international standard for HBeAg assay calibration lies in the genomic organization of the virus and mutations.

The new WHO 2024 recommendations have simplified the clinical algorithm for the diagnosis, treatment and monitoring of HBV and expanded the criteria for starting treatment. The new recommendations on treatment initiation criteria take into account about 50% of HBsAg positive people compared to 15% to 25% previously [1] [2]. They consider people of all ages, including pregnant women, and this allows for a common entry point for access to treatment in all age groups and populations. Key updates to the new recommendations indicate that all adults and adolescents over 12 years of age should be treated with a viral load greater than 2000 IU/mL and a higher-than-normal ALT level [2]. A person with abnormal ALT persistence and a viral load less than 2000 IU/mL and who does not have comorbidities should be put on treatment [2]. In the absence of viral load, which has long been a major barrier to access to treatment, it is recommended that all individuals with persistent abnormal ALT only be put on treatment [1] [2]. Persistence of abnormal ALT is defined as at least two ALT values below or above baseline over a period of 6 to 12 months [1]. Most WHO member countries have now developed national plans, policies and strategies to combat viral hepatitis. In

Burkina Faso, according to standards and protocol for the management of viral hepatitis developed in September 2019, treatment is indicated for anyone over 25 years of age with a viral load greater than 20,000 IU/mL and for anyone with persistent abnormal ALT with or without HBV-DNA [15]. Treatments are still centralized at the level of the Central Purchasing Office for Generic Medicines, which does not allow for a simplified health service, thus making it difficult for affected populations to access treatment.

This study has some limitations: The first is that we did not measure ALT in our samples. This would allow us to better appreciate the WHO recommendations on the HBeAg/ALT combination and ALT only for the treatment initiation criteria. The second limitation is the absence of the pre-core mutant research. In fact, HBV genome is a relaxed circular DNA (rcDNA) with one complete negative strand and one incomplete positive strand. It encodes four overlapping open reading frames (ORFs), which allow the synthesis of viral proteins [16] [17]. Consequently, a mutation in a specific region of the genome can influence the synthesis of proteins. There are mutations in the pre-C/C gene that influence the production of HBeAg without affecting viral replication [6] [7]. The G1896A substitution results in the emergence of a premature stop codon, which ultimately leads to the termination of HBeAg translation without influencing viral replication [6].

5. Conclusion

The results demonstrated that there was no statistically significant discrepancy between the proportions of individuals eligible for treatment on the basis of HBeAg (5.5%) and VL (7.8%). This suggests that the WHO recommendation on the use of HBeAg in combination with ALT would be reasonable in resource-limited countries. It is therefore recommended that HBeAg be tested using fourth-generation ELISAs or their variants. The data on the diagnostic performance of the ELFA VIDAS HBe/anti-HBe kit versus PRC for HBV DNA quantification demonstrated low sensitivity, with figures ranging from 18.51% to 75.00% for DNA quantities above 2000 IU/ml and 2,000,000 IU/ml, respectively.

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Author's Contributions

The study was conceived and designed by AD, AMS and MKG. AD, JSB, and MNGO conducted laboratory investigations, acquired and curated the data. AD performed the analysis and interpretation of the data. AD, AMS and MKG wrote the original draft of the manuscript. All authors contributed to the critical review of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations and Acronyms

DNA	Deoxyribonucleic Acid
WHO	World Health Organization
ALT	Alanine Aminotransferase
ELFA	Enzyme Linked Fluorescent Assay
IRSS-DRO	Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest
VL	Viral Load
HCC	Hepatocellular Carcinoma
CI	Confidence Interval
PPV	Positive Predictive Value
NPV	Negative Predictive Value
HBV	Hepatitis B Virus
RDT	Rapid Diagnostic Test