Prevalence of Hepatitis B Virus Infection Markers among Patients of the Ibn Sina University Hospital Center (Rabat, Morocco)

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Keywords
Prevalence · Hepatitis B virus · Serological markers · Morocco

Abstract
Introduction: Viral hepatitis B is a global scourge affecting millions of people worldwide. In Morocco, hepatitis B is considered a public health problem, and available data converge to consider Morocco as a country with intermediate endemicity. In the present study, we have planned to evaluate the HBV prevalence in Morocco on a large scale and to assess the prevalence of different serological markers for better management of this infection in Morocco. Methods: This study was conducted on 18,877 patients referring to the Ibn Sina University Hospital Center of Rabat, Morocco. HBV serological markers including HBsAg, HBsAb, HBeAg, HBeAb, and total HBcAb were assessed by immune-enzymatic assays. The quantification of HBV DNA was performed by real-time PCR. Results: The overall prevalence of positive cases for HBsAg, HBsAb, and total HBcAb was 2.47%, 27.66%, and 21.2%, respectively. From 141 patients with an isolated HBcAb serological profile (HBcAb+/HBsAb−/HBsAg−), HBV DNA was detected in 10 patients, representing a rate of 7.09%. In the present study, up to 95.78% of HBV chronic carriers were negative for HBeAg. Conclusion: This study highlights a higher prevalence of HBsAg in the hospital-based population than the general population reported previously in Morocco and a very low HBV immunization coverage. Of particular interest, detectable HBV DNA levels in isolated HBcAb patients show that exclusive HBsAg screening cannot eliminate the risk of HBV transmission in certain cases. Many efforts are then mandatory to promote serological testing and increase the vaccination rate to limit viral dissemination for better management of this disease in Morocco.
Hepatitis B Status in Rabat, Morocco

Samples from 10 different hospitals. Patients are mainly from the Rabat region but also from various cities of the country coming for medical care, surgeries, consultation, tertiary healthcare, etc. Samples were sent to the Virology Lab with a medical prescription to carry out HBV detection, viral load assessment, and virus genotyping, as a part of several diagnoses that will be used by the medical staff for better management of patients’ respective diseases. The study was conducted under the local ethical rules, and informed consent was obtained from all patients.

Clinical Specimens
Specimens were recruited between March 2015 and February 2018. Blood samples have been collected on serum-separating tubes for serological tests and EDTA tubes to quantify HBV DNA by real-time PCR. HBV status was performed without any presumption of hepatitis infection.

HBV Serological Assays
HBV serological markers, notably HBsAg, HBeAg, HBsAb, and HBcAb, were detected by Chemiluminescent microparticle immunoassay on the Architect i2000SR analyzer (RRID: SCR_010477; Abbott) according to the manufacturer’s recommendations. The results are expressed in international units per milliliter for HBsAg and S/CO ratio for the other markers. An anti-HBs rate of 10 IU/L was considered protective. The HBeAb were detected by a competitive reaction. HBsAg-positive samples with an S/CO ratio between 1 and 1,000 were confirmed by the neutralization reaction on the Architect i1000SR instrument as recommended by the manufacturer (RRID: SCR_010477; Abbott).

Quantification of HBV DNA
The quantification of HBV DNA was performed by real-time PCR on the Abbott m2000 system (RRID: SCR_010477; Abbott). HBV DNA was extracted from plasma samples by using m2000sp during sample preparation. Quantification of HBV DNA extracted was carried out by using m2000rt. The lower limit of detection was 10 IU/mL. Samples were considered HBV DNA positive if the viral loads are higher than or equal to 10 IU/mL.

Materials and Methods

Study Setting
The study was conducted in the Central Virology Laboratory, referring to Rabat’s Ibn Sina University Hospital Center, receiving samples from 10 different hospitals. Patients are mainly from the

Prevalence of HBsAg, HBeAg, and Total HBC
Among the recruited patients, 466 patients were HBsAg reactive (53% males, 41% females, and 6% children), representing a prevalence of 2.47%. HBeAg were detected in 5,222 patients (27.66%) and total HBC in 4,002 patients (21.2%), whereas 12,335 of recruited patients were HBV free (65.34%). Based on these serological results, pa-
Patients were classified into 6 serological profiles (Table 1): susceptible, immune due to natural infection, and immune due to hepatitis B vaccination profiles prevailed (Table 2).

**HBV DNA in Patients with Isolated HBcAb**

In this study, HBV DNA was investigated by qPCR in 141 among the 870 patients with an isolated HBcAb profile (HBsAg−/HBsAb−/HBcAb+). Overall, 92.91% of cases had undetectable viral DNA load (131/141). In contrast, 10 patients were HBV DNA positive, and the viral load ranged between 10 and 708 IU/mL, with a median of 34.97 IU/mL and a mean of 120.86 ± 213.76 IU/mL.

**Prevalence of HBeAg in HBV Chronic Carriers**

Among the 466 HBsAg-positive cases, 142 were HBV chronic carriers as they exhibited HBsAg positivity beyond 6 months. HBeAg was detected in only 6/142 cases representing a rate of 4.23%, while their HBeAb were negative. In contrast, the remaining 136 patients had negative HBeAg and positive HBeAb (Table 3). Among them, 70.42% (100/142) had a viral load <2,000 UI/mL with a median of 244.39 IU/mL, ranging between 11 and 1,950 IU/mL (mean: 498.64 ± 532.24 IU/mL) while the remaining 36 patients had a viral load >2,000 UI/mL, and the median was 22,159 IU/mL, ranging from 2,120 to 6.24 × 10^8 IU/mL (mean 1.9 × 10^7 ± 1.03 × 10^8 UI/mL).
**Discussion**

Despite the large efforts made to fight against HBV infection, hepatitis is still a major health problem in Morocco. In this study, the prevalence of HBsAg was 2.47%, which is in line with the overall prevalence estimated at 3.3% by the WHO in the Eastern Mediterranean Region. This HBV seroprevalence consolidates the classification of Morocco as an endemic middle area with a prevalence between 2 and 8% [7–9]. Previous studies conducted in Morocco on the general population have reported HBV prevalence <2%: 1.66% among the active population [10] and 1.81% in the cross-sectional survey enrolling in the large screening program for hepatitis B and C conducted by the Pasteur Institute of Morocco [11]. Other studies conducted on blood donors in Rabat in 2013 and 2016 have reported the prevalence of 0.8 and 1.34%, respectively [12, 13], suggesting a slight difference in HBsAg prevalence between our recruited patients and the general populations as reported previously.

Overall, only 27.66% of patients were positive for HBsAb. This antibody screening is carried out to follow the evolution of hepatitis B to check HBsAg/HBsAb seroconversion status, but also with the aim of evaluating the effectiveness of HBV vaccination. This value remains very low given the development achieved in Morocco in terms of immunization coverage since 1999 [14]. Of particular interest, a significant portion of patients recruited for this study are at high risk of contracting HBV as they are likely to receive massive and/or iterative transfusions (hemophiliacs, dialysis, renal failure, and organ transplant candidates), in psychiatric institutions, hospitalized patients, etc. The double burden due to sur-infection with HBV could be fatal for these vulnerable patients. Therefore, vaccination is still the fundamental cornerstone to fight against HBV, as it prevents chronic B viral hepatitis in 95% of cases and limits the occurrence of hepatocellular carcinoma [15]. In Morocco, anti-HBV vaccination had started in the 2000s, and vaccination coverage for children under 1 year has increased from 33% in 2000 to 93% in 2005 [14]; therefore, we can easily assume that in the upcoming years, most adults will be vaccinated leading to a significant decrease in HBV-positive cases. Total HbcAb were found in 21.2% of patients. These antibody compounds are IgM HbcAb and IgG HbcAb, which are widely reported as a good indicator of the HBV endemic status. A lower prevalence of total HbcAb was found in low-prevalence countries such as Iran, France, and Spain (4.9%, 7.3%, and 8.2%, respectively) [16–18]. In contrast, a higher prevalence was found in high-prevalence countries like Nigeria, Togo, and Mauritania (32%, 53.9%, and 76.5%, respectively) [19–21].

Assessment of HBsAg, HBsAb, and total HbcAb serological markers of HBV is of great interest in hepatitis management as they allow the identification of different phases of HBV infection as well as monitoring of patients infected by HBV. The other virological markers, notably IgM HbcAb, HbeAg, HbeAb, quantitative HBsAg, and molecular HBV DNA quantification, are widely used but depend on the initial results of the first 3 markers. Ultimately, and according to the obtained results, the patients had 6 distinct serological profiles (Table 2).

The great majority of patients has never been in contact with the HBV (65.34%); HBsAg-positive patients (HBsAg+/HBsAb−/HbcAb+) represent 2.38% of total recruited patients, and this serological profile is referring to an ongoing HBV infection (acute or chronic). Chronically infected patients are characterized by the persistence of HBsAg beyond 6 months and the loss of IgM HbcAb, while the acute infection is distinguished by positive IgM HbcAb or by the absence of detectable total HbcAb in the case of recent infection. Another atypical serological profile was found with a very low rate (0.09%), and it is characterized by the positivity of the 3 serological markers (HBsAg+/HBsAb+/HbcAb+). This situation usually indicates ongoing HBsAg/HbsAb seroconversion but does not exclude chronic infection with simultaneous detection of HBsAg and HBsAb. This serological profile can be explained by the emergence of HBV escape mutants not
recognized by the circulating HBsAb directed against the wild-type viruses or by the presence of heterologous subtype-specific antibodies directed against HBsAg subtypes different from the coexisting HBsAg [22]. Patients immunized by HBV infection (HBsAg−/HBsAb+/HbcAb+) represent a rate of 14.12%. This serological profile characterizes patients formerly infected, recovered, and immunized against HBV. Last, immunized patients by vaccination (HBsAg−/HBsAb+/HbcAb−) were present with a proportion of 13.46%.

An isolated HbcAb serological profile (HBsAg−/HBsAb−/HbcAb+) was observed in 4.61% of recruited patients. These antibodies are normally associated with HBsAg during acute and chronic hepatitis B or HBsAg after HBsAg anti-HBs seroconversion. This serological profile could be explained by [23–25] the following: (1) the loss of HBsAg after the resolution of HBV infection; indeed, the disappearance of HBsAb may occur several years after HBsAg anti-HBs seroconversion, while HbcAb persist throughout life; the persistence of these antibodies is probably due to their high level of immunogenicity [26]; (2) acute hepatitis B during the so-called acute window period before the appearance of HBsAb; (3) false-positive reactivity for total HbcAb; (4) this serological profile may also correspond to occult B viral hepatitis. In this study, HBV DNA was detected in 7.09% of patients with isolated HbcAb. The absence of detectable HBsAg in the serum of these patients is probably due to the production of an antigenically modified S protein (mutants S) and therefore not detected by conventional serological tests [25]. In several studies, detection of HBV DNA in serum “HbcAb isolated” has been reported in several studies, notably in Tunisia, Lebanon, and Egypt with a prevalence of 4.47%, 13%, and 17.2%, respectively [23, 27, 28]. According to the literature, the occurrence of detectable HBV DNA among patients with an “isolated HbcAb” serological profile becomes higher when they are co-infected with HCV and/or HIV [29, 30]. In this regard, this serological profile should be monitored closely as it may entail the HBV transmission, especially in patients receiving blood transfusions or organ transplants.

In this study, only 4.23% of HBV chronic carriers were HBeAg positive. These results are in agreement with those reported in other African countries, including Cameroon where a survey conducted in 3 hospitals in Yaoundé showed that 92% of the chronic carriers of hepatitis B were negative for HBeAg [31]. Scientific evidence showed that quantification of HBV DNA in patients with chronic HBV infection and negative HBeAg is of great interest in hepatitis management as it leads to differentiation between 2 phases of chronic hepatitis B. Patients with low HBV DNA load (<2,000 IU/mL) correspond to HBeAg-negative chronic HBV infection (formerly known as inactive carrier phase) that may allow the resolution of hepatitis B (HBsAg anti-HBs seroconversion) or reactivation episodes, while patients with a high viral load (≥20,000 IU/mL) match with HBeAg-negative chronic hepatitis B phase and are most likely to carry HBV variants [32]. HBeAg-negative chronic hepatitis B is frequently observed in patients in sub-Saharan Africa, the Middle East, and the Mediterranean basin. Several studies reported that this form progresses gradually and has become the dominant form of chronic B hepatitis around the world. An increase in the rate of negative HBeAg patients in the USA and countries of Europe and Asia is reported [33]. Of particular interest, HBeAg-negative chronic hepatitis B is widely reported to be associated with the development of severe liver disease [32, 34–36]. Undetectable level of HBeAg in chronic hepatitis B patients was reported to be a result of 2 major mutations: pre-core mutation characterized by the substitution of guanine (TGG) by adenine (TAG) in position 1,896, resulting in premature cessation of protein synthesis, and the double mutation of PBC (A1762T and G1764A) responsible for a significant decrease in the expression of HBe protein. These mutant forms allow the virus to better resist the pressure of the host immune system [37].

Moreover, many previous studies have reported that pre-core mutation is one of the main causes of the negativity of HBeAg in chronic hepatitis B patients. Of particular interest, these studies reveal the predominance of genotype D among their patients [34, 38, 39]. Indeed, pre-core mutation (G1896A) helps to stabilize the stem-loop of pre-genome RNA by strengthening the pairing with thymine located in position 1,858 in genotypes B, D, E, and C in contrast to genotypes A, F, and H harboring a cytosine in position 1,858 and requiring a second mutation (C1858T) to stabilize the pre-genome RNA [37, 40].

In Morocco, recent studies among patients with chronic hepatitis B reported the predominance of genotype D with percentages varying between 87.6 and 100%. These results could explain the high prevalence of HBeAg-negative patients found in this study [39, 41–43]. Hence, a close follow-up is needed for better management of these patients.

The present study is very informative and has many strengths, namely, the huge number of recruited cases and the evaluation of HBV status based on a considerable number of virological biomarkers. However, the main limitation of the study is the nonrepresentativeness of re-
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M.F. participated in the project design, experimental analysis, and statistical analysis and drafted the manuscript; H.K. contributed to the interpretation of the results; M.E.M. provided critical feedback and data analysis and reviewed the final manuscript; B.B. participated in data analysis and review of the final manuscript; N.B. participated in the experimental analysis; A.F.-M. participated in the design of the project and review of the final manuscript; M.S. participated in the design and coordination of the project and review of the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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Author Contributions

M.F. participated in the project design, experimental analysis, and statistical analysis and drafted the manuscript; H.K. contributed to the interpretation of the results; M.E.M. provided critical feedback and data analysis and reviewed the final manuscript; B.B. participated in data analysis and review of the final manuscript; N.B. participated in the experimental analysis; A.F.-M. participated in the design of the project and review of the final manuscript; M.S. participated in the design and coordination of the project and review of the final manuscript.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Statement of Ethics

This study was conducted in the Virology Lab of the Ibn Sina University Hospital Center which is a central and a referant lab receiving samples for all the public hospitals in Rabat, Morocco. All samples were sent with a medical prescription to carry out HBV detection, viral load assessment, and virus genotyping, and all patients provided their written and informed consent for this test and have agreed to do these analyses. Moreover, all these analyses were done for hepatitis diagnosis, and therefore we did not seek ethical approval to use these samples for research purposes and was not required for this study in accordance with local/national guidelines. The publication of these results was approved by the Scientific Board of the Central Virology Lab.

Conclusion

This study clearly highlighted a high level of HBV prevalence in Morocco and a very low HBV immunization coverage. HBV infection is still a public health problem, and new recommendations, in accordance with WHO guidelines, should be set to promote serological testing and reinforce the vaccination protocol to limit viral dissemination and ensure better management of this disease in Morocco.

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