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Immune-pathological phases of chronic Hepatitis B infections among Sudanese individuals towards personalization of management

Abstract

Background: Hepatitis B virus (HBV) infection is an immune liver disease affecting millions worldwide. Despite availability of an efficacious vaccine, elimination of HBV infections is aloft. This study aimed to identify the immune-pathological phases of chronic HBV infection (CHBV) among Sudanese individuals to refine management strategies.

Materials and methods: In a prospective cross-sectional study and following informed consent, 1593 individuals with HBs Ag reactivity were enrolled. Serum total protein/albumin, ALT, AST, total bilirubin, HBs Ag/Ab, HBe Ag/Ab, HBc IgM/HBc total antibodies and HBV viral loads were measured.

Results: Mean aminotransferase levels for HBs Ag-reactive individuals were significantly higher compared to apparently normal individuals, while the mean total protein and serum albumin were within normal ranges. The majority of HBs Ag-reactive individuals were reactive to total anti-HBc and HBe Ab, while concurrent HBe Ag/Ab reactivity was seen in a minority. Inactive carriers constituted the majority of HBs Ag reactive individuals, while the immune tolerance CHBV phase could not be identified. The reactivation phase had the highest viral load.

Conclusion: inactive carrier state is the predominant immune-pathological phase among Chronic HBV Sudanese individuals. Regular follow ups and no oral anti-viral drug treatment as the management of choice to reduce cost, drug-associated toxicities and emergence of resistant strains.

Keywords: CHBV infections, immuno-pathological phases, immune tolerance

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Abbreviations: HBV, Hepatitis B virus; rcDNA, double-stranded relaxed circular DNA; CHBV, chronic Hepatitis B virus

Introduction

Hepatitis B virus (HBV) infection is a major global public health problem, with overlapping modes of transmission. HBV is one of the smallest enveloped animal viruses with a virion diameter of 42 nm; the particles are not infectious and are composed of lipids and proteins that form part of the surface of the virion. The nucleo-capsid structure of HBV contains a genome (3.2 kb in length) of partially double-stranded relaxed circular DNA (rcDNA) molecule and a polymerase enzyme. The outer capsule contains HBs Ag, while the inner core contains HBc and HBe Ags. The virus is not directly hepatotoxic, but the host's immune responses are thought to be the cause of the liver injury. Cellular rather than humoral immune responses are primarily involved in the disease pathogenesis.[1–5] WHO has estimated that globally around 240 million people are chronically infected with HBV with 500,000-700,000 deaths annually. Sudan is a country with high HBV sero-prevalence of greater than 8% and HBs Ag reactivity of 16%–20% in the general population.[6–9] Chronic hepatitis B virus (CHBV) infection is a highly heterogeneous disease regarding the levels of virus replication, liver disease activity and humoral responses. Symptomatic and asymptomatic forms of acute and chronic infections may be discovered incidentally through laboratory assays of viral markers. Viral markers may occur singly or in various combinations depending on the natural history of infection. The most common outcome after infection is the expression of diverse serological markers of varying clinical and epidemiological significances namely: HBs Ag, HBs Ab, HBc IgM, HBc Ab, HBe Ag, HBe Ab. Detection of these antigenic and serological markers helps to determine immune pathological phases of CHBV infection and help plan better management strategies. Immune tolerance phase of CHBV is always depicted as the early that is characterized by high HBV DNA load, HBe Ag reactivity and normal alanine transferase (ALT) level. The presence of immune tolerance phase has recently been challenged. In addition, some studies have documented a low but persistent immune destruction of infected hepatocytes by low-level cytotoxic T lymphocyte infiltration.[1,10–12] During the inactive carrier phase of CHBV no further damage to the liver occurs, with a chance of conversion to immune clearance, reactivation or immune control phases. Most inactive carriers usually have low DNA load, HBe Ag negativity and normal ALT levels. The third CHBV phase is an immune clearance (activation) phase where host immune system becomes mature and begins to recognize HBV-related epitopes on hepatocytes with immune-mediated viral clearance and hepatocytes damage. This phase usually lasts from several months to many years with HBe Ag positivity, high HBV DNA loads, elevated serum aminotransferase levels and active inflammation of the liver. Reactivation phase usually ensues on 20%-30% per cent of inactive carriers during follow up. This phase is usually asymptomatic but can occasionally mimic acute viral hepatitis where appropriate diagnosis and management becomes a necessity.[13–18] This study aimed to delineate the immune-pathological patterns of CHBV among Sudanese individuals to inform guidelines of management to prevent drug toxicities and reduce cost.

Materials and methods

Study design

This was a prospective, cross-sectional, analytical and facility-based study.

Ethical considerations

The proposal was reviewed and approved by the Scientific and Ethics Committees of the Institute of Endemic Diseases, University of Khartoum. Apparently healthy volunteers and HBs Ag reactive individuals were individually consented and asked to sign a consent form.

Study sites

The study was conducted at the Central Blood Bank, Khartoum, Sudan and Alzahrawi Medical Laboratory [a central referral laboratory, Khartoum] and the Department of Clinical Pathology and Immunology, Institute of Endemic Diseases, University of Khartoum, Sudan.

Study population

Individuals with HBs Ag reactivity and apparently healthy volunteers were recruited. Individuals with concurrent HIV or HCV serological reactivity and those who refuse/fail to give an informed consent were excluded.

Blood samples, biochemical and viral serological markers

Ten mls of bloods were collected for biochemical, immunological and viral loads analyses. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total protein/albumin, total bilirubin were estimated using an automated chemistry analyzer (Biosystem BTS_{310}, Biosystem, Barcelona, Spain). HBs Ag, anti-HBs Ab, HBe Ag, anti-HBe, total anti-HBc and Ig M anti-HBc were detected using commercially ELISA kits (One-step incubation, competition principle) [Atlas Link Technology Co. Ltd. Beijing, China].

Molecular detection and quantification [viral load] of HBV by qPCR

HBV DNA was extracted from 3 mls of EDTA blood using a modified Guanidine method. [Pramanick et al. 1976]. Briefly: 10 mls of red cell lysis buffer (1mM NH4HCO3+115mM NH4Cl) were added to the whole blood, mixed well and centrifuged for 5 minutes at 4000 rpm and repeated until a clear pellet of white blood cells appeared at the bottom of the tube. Two mls of white blood cells (WBCs) lysis buffer (10mMTris-HCl+0.1mM EDTA+100mMNaCl+20%SDS), 10µl of proteinase K (10mg/ml), 300 µl of ammonium acetate (MW=57.81g/100ml ddH2O) and 1 ml of guanidine (4 M/ 250 ml ddH2O) were added to the pellets. The mixture was incubated overnight in a water-bath at 37 °C. Two mls of chloroform were added and the upper layer was aspirated into a new tube. Ten mls of ice-cold ethanol was added to the upper layer, mixed well and centrifuged for 5 minutes at 4000 rpm. The tubes were vertically dried and the pellet was washed by 4 mls of cold 70% ethanol. The resulting pellets were allowed to dry and were re-suspended in RNase/DNA-ase-free water. The DNA concentration was measured using Nanophotometer® P300 (IMPLEN GmbH, Munich, Germany) and stored at –20°C until analyzed.

Viral load determination

Molecular detection and quantification of HBV were done using commercial kits [Genesig, Primerdesign, UK] with the following primers and probes (FW: 5′AGCGTCTAGCCATGGCGTT3′ RV: 5′GCAAGCACCCTATCAGGCAGT3′ Probe: FAM 5′TCTGCGGAACCGGTGAGT3′ MGB NFQ. FAM as a reporter dye attached to the 5′ end of the primer, a non-fluorescent quencher (NFQ) and minor groove binder (MGB) were attached to the 3′ end of the primer. To determine samples viral loads, a standard curve was constructed from serial dilutions of the positive control [cloned plasmids containing inserts in the conserved region of HBV virus] in an initial concentration of 4.9×10^9 IU/mL and total of eight points in each curve. The amplified product was detected with the use of fluorescent dyes that are linked to oligonucleotide probes, which bind specifically to the amplified product during thermo-cycling. Negative controls were included in all reactions to test kit stability, while internal controls were used to rule out possible false results, monitor all stages of the analysis and reveal the effect of PCR inhibitors. The reactions were performed in Spectrum48 qPCR machine (ESCO Micro Pte Ltd, Singapore).

Statistical analysis

Statistical analysis was performed using the shareware Epi Info version 7. Standard descriptive statistical analysis and correlations between variables [using *t-test*] was carried out for biochemical parameters and HBV viral loads. P-value <0.05 was considered significant.

Results

A total of 1593 individuals with HBs Ag reactivity and 300 apparently healthy individuals with HBs Ag non-reactivity (comparators) were enrolled. The mean age was 35.4±11.6 years and 32.00±9.9 years for the hepatitis B surface antigen positive and comparator groups respectively. The male: female ratio was 3:1 and 1:1 in the hepatitis B surface antigen positive and the comparator groups respectively. Male patients were more frequent compared to females in the HBs Ag positive group (p=0.0001). The commonest age group among infected individuals was the 28-37 years. Mean aminotransferase levels for HBs Ag reactive individuals were significantly higher compared to comparators (ALT: 20.8±37 IU/L and 10.5±6.2 IU/L; p=0.004) (AST: 37.0±124.1 IU/L and 13.5±7.2 IU/L; p=0.007). Mean total proteins and serum albumin, were comparable in both groups (Table 1). Hbe Ag reactive individuals (4.9%, 78/1593) had significantly higher aminotransferases, total and direct bilirubins compared to Hbe Ag non-reactive individuals (p=0.001) (Table 2). The majority (77.7%; 1238/1593) of the HBs Ag reactive individuals were reactive to total anti-HBc antibodies, while a small percentage [2%; 32/1593] was HBs Ag+/HBe Ag+ and Ig M anti-HBc reactive. The majority (80.0%, 1274/1593) of HBs Ag+ had HBe Ab, HBe Ag and concurrent Hbe Ag/HBe Ab reactivity was seen in a minority (4.9%, 78/1593; 6.0%, (98/1593). Fourteen per cent (221/1593) of HBs Ag reactive individuals showed no Hbe Ag/HBe Ab reactivity, while anti-HBs was seen in a minority (2.9%, 46/1593). No individuals fitted the criteria for immune tolerance, while immune clearance, reactivation and inactive carriers phases were seen in 2.2% (35/1593), 1.3% (20/1593) and 96.5% (1537/1593) respectively. The mean viral load for HBs Ag reactive individuals was 520±809 IU/ml, while those in the reactivation immune pathological pattern had the highest viral load (1495.0±2755) compared to the immune clearance and the inactive carrier groups (451±250 and 437±399.0 respectively) (p=0.0001).

Table 1 Mean age, aminotransferases levels, total proteins/albumin and bilirubin in HBs Ag positive and HBsAg Negative apparently healthy individuals [Comparators]

Variables	HBs Ag reactive individuals (n=1593)	Comparators [apparently healthy] (n=300)	*p*-value
Mean age/years±SD	35.4±11.6	32.00±9.9	0.4
ALT (IU/ml)	20.6±36.1	9.6±5.9	0.008*
AST (IU/ml)	31.2±94.0	12.3±6.8	0.001*
Total Proteins (grams/dL)	07.0±0.8	07.8±6.8	0.06
Albumin (grams/dL)	03.8 ±0.7	03.9±0.8	0.9
Total Bilirubin (mg/dL)	0.70±1.8	0.459±0.2	0.1
Direct Bilirubin (mg/dL)	0.3±1.3	0.14±0.04	0.3
HBe Ag+	4.9% (78/1593	___	____
HBe Ab+	67% (1070/1593)	___	____
Total HBc Ab	77.7% (1238/1593)	___	____

Continuous variables are expressed as mean±SD *statistically significant

Table 2 Mean ages, aminotransferases levels, total proteins/albumin and Bilirubin levels in HBe Ag reactive

Variables	HBe Ag reactive (n=78)	HBe Ag non-reactive (n=1515)	*p*-value
Mean age/years±SD	35.4±11.6	32.00±9.9	0.4
ALT (IU/ml)	127.2±205.2	15.2±9.3	0.001*
AST (IU/ml)	336.7±543.3	19.9±10.6	0.001*
Total Proteins (grams/dL)	07.3±0.8	07.2±3.7	0.9
Albumin (grams/dL)	03.7±0.8	03.9±0.7	0.5
Total Bilirubin (mg/dL)	05.6±10.5	0.6±0.3	0.001*
Direct Bilirubin (mg/dL)	04.0±7.9	0.093±0.005	0.001*

Continuous variables are expressed as mean±SD, *statistically significant

Discussion

Hepatitis B virus infection is among the most neglected diseases with a considerable mortality risk worldwide, the African and Western Pacific regions accounted for the majority of cases. Despite the availability of an efficacious vaccine and a number of anti-viral drugs, the elimination of HBV infection is far from achievable. This is due to persistent cases of CHBV cases that continuously feed the pool of new HBV cases. The dilemma to treat patients with CHBV is ever brewing and patients have to be advised wisely to save money, prevent drug toxicity and emergence of drug-resistance. The choice is between: no treatment with regular follow ups and treatment with a limited period of IFNα or life-long use of oral nucleo(s) tides analogues with mounting cost/toxic effects/induction of drug resistance.

Understanding the genotypes of HBV and the immune pathological patterns of CHBV are very important to modify management strategies, a right step towards treatment personalization. Triaging patients to treatment or no treatment has to be based on genotyping HBV and delineation of immune pathological patterns CHBV. It is well documented that HBe Ag negative chronic carriers have reduced sustained responses to nucleoside/nucleotide analogues. It has also been reported that IFNα is less effective against HBV genotypes D and C and that lamivudine resistance is higher among patients harboring HBV genotype A. Nucleosides/nucleotides analogues singly or in combination could lead to measurable virological and biochemical response, but does not significantly suppress the development of hepatocellular carcinoma.[3,19–27]

The commonest age group affected in our study cohort is younger than that reported from the Far East, where the patients were in their late forties. The predominance of males, may point to the fact that males are probably more affected than females, concordant with results for other regions. On the other hand, this probably indicates female's decreased treatment seeking attitudes in developing communities. The majority of HBs Ag reactive Sudanese individuals were HBc Ab

reactive, less than that reported from other regions, which indicates that HBV immunity is mostly acquired through viral exposure in the community.[23,28] A small per cent age of the study individuals were Hbe Ag reactive with significantly higher aminotransferases/ total and direct bilirubins levels compared to Hbe Ag non-reactive individuals. This probably indicates increased disease activity and viral replication. A small per cent age of the study cohort were, HBs Ag+/Hbe Ag+/HBc IgM reactive probably indicating acute infection within the recent 6-8 months. These individuals will either go to develop anti HBs and anti-HBc IgG with elimination of the virus and be cured. Alternatively, anti-Hbc IgM will disappear with appearance of anti-HBc IgG antibodies and persistence of the infection (chronic inactive carrier). Reports were very variable about the clinical significance of IgM/Ig G anti-HBc antibodies as markers for virus replication.[29–32] HBe Ag was seen in a small per cent ages of HBs Ag+ individuals, while the majority of our patients were HBe Ab reactive. Concurrent Hbs Ag/Hbe Ag and their corresponding antibodies is well documented. CHBV with low levels of HBs Ag have low levels of viral DNA i.e. low replication as was seen in a small per cent age of the study cohort.[33] Cheng and colleagues[34] reported that the majority of their patients were Hbs Ag/HBe Ag/Hbc Ag reactive, a finding that is discordant with our findings.[34] Hbe Ag/HBe Ab non-reactivity was seen in the majority of our cohort, this probably indicates disease inactivity or immune exhaustion that has been well-documented in CHBV. Furthermore, Hbe Ag/HBe Ab non-reactivity could mean mutations in the pre-core or core regions of the viral genome. A small numbers of the study cohort showed co-existence of HBs Ag/Hbs Ab reactive probably on a transitional state to clear the virus or probably suggesting a selection of immune escape mutants during the disease period, a finding previously reported.[32,34–37] Individuals with immune tolerance CHBV could not be identified within the study cohort, in agreement with recent reports that challenged the early concepts of existence of this immune pathologic phase. Recent evidence showed that subtle liver damage in the presence of normal aminotransferases levels exits in CHBV individuals.[10,32,34–37]

Conclusion

In conclusion, inactive carrier state is the predominant immune-pathological phase among Chronic HBV Sudanese individuals. Regular follow ups and no anti-viral drug treatment should be advocated as the management of choice to reduce cost, drug-associated toxicities and emergence of resistant strains.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Dimitropoulou D, Karakantza M, Theodorou GL, et al. Serum cytokine profile in patients with hepatitis B e antigen-negative chronic active hepatitis B and inactive hepatitis B virus carriers. *World J Gastrointest Pathophysiol*. 2013;4(1):24–27.
2. Hwang JP, ASF Lok. Management of patients with hepatitis B who require immunosuppressive therapy. *Nat Rev Gastroenterol Hepatol*. 2014;11(4): 209–219.
3. Fanning GC, Zoulim F, Hou J, et al. Therapeutic strategies for hepatitis B virus infection: towards a cure. *Nat Rev Drug Discov*. 2019;18(11):827–844.
4. Virüsü HB, Siklusu Y, İnan N, et al. Hepatitis B Virus : Biology and Life. 2019.
5. Wang B, Mufti G, Agarwal K. Reactivation of hepatitis B virus infection in patients with hematologic disorders. *Haematologica*. 2019;104(3):435–443.
6. Indolfi G, Easterbrook P, Dusheiko G, et al. Hepatitis B virus infection in children and adolescents. *Lancet Gastroenterol Hepatol*. 2019;4(6):466–476.
7. Elduma H, Saeed NS. Hepatitis B virus infection among staff in three hospitals in Khartoum, Sudan, 2006-07. *East Mediterr Health J*. 2011;17(6):474–478.
8. Mudawi HM. Epidemiology of viral hepatitis in Sudan. *Clin Exp Gastroenterol*. 2008;1: 9–13.
9. Hope VD, Eramova I, Capurro D. Prevalence and estimation of hepatitis B and C infections in the WHO European Region : a review of data focusing on the countries outside the European Union and the European Free Trade Association. *Epidemiol Infect*. 2014;142(2):270–286.
10. Noor B, Chowdhury RS. Factors Affecting Knowledge & Consciousness of Bangladeshi People about Hepatitis B1 (HB1): An Application of Linear Logistic Regression. American J Epidemiol Infecti Dis. 2015;3(3):70–75.
11. Bertoletti A, Kennedy PT. The immune tolerant phase of chronic HBV infection: new perspectives on an old concept. *Cell Mol Immunol*. 2014;12(3): 258–263.
12. Mbaawuaga EM, Iroegbu CU, Ike AC. Hepatitis B Virus (HBV) Serological Patterns in Benue State, Nigeria. Open J Med Microbiol. 2014;4(1):1–10.
13. Wong GL. Management of chronic hepatitis B patients in immune tolerant phase: what latest guidelines recommend. *Clin Mol Hepatol*. 2018;24:108–113.
14. Shi Y, Shi C. Molecular characteristics and stages of chronic hepatitis B virus infection. *World J Gastroenterol*. 2009;15(25):3099–3105.
15. Ni Y-H, Chang M-H, Wu J-F, et al. Minimization of hepatitis B infection by a 25-year universal vaccination program. *J Hepatol*. 2012;57(4):730–735.
16. Bhopale GM. Pathogenesis of Hepatitis B Virus. *Int J Curr Microbiol App Sci*. 2016;5(6):619–626.
17. Dong J, Yang X, Wang L, et al. Modulation of Tim-3 Expression by Antigen-Dependent and -Independent Factors on T Cells from Patients with Chronic Hepatitis B Virus Infection. *Front Cell Infect Mi*. 2017;7:1–10.
18. Wang Q, Pan W, Liu Y, et al. Hepatitis B Virus-Specific CD8+ T Cells Maintain Functional Exhaustion after Antigen Re-exposure in an Acute Activation Immune Environment. *Front Immunol*. 2018;9:219.
19. Lok AS-F. Hepatitis B Treatment: What We Know Now and What Remains to Be Researched. *Hepatol Commun*. 2019;3(1):8–19.
20. Marcellin P, Lau GKK, Bonino F, et al. Peginterferon Alfa-2a Alone, Lamivudine Alone, and the Two in Combination in Patients with HBeAg-Negative Chronic Hepatitis B. *N Engl J Med*. 2018;351:1206–1121.
21. Enomoto M, Tamori A, Nishiguchi S. Hepatitis B virus genotypes and response to antiviral therapy. *Clin Lab*. 2006;52(1-2):43-47.

22. Palumbo E, Scotto G, Faleo G, et al. Prevalence of HBV genotypes in South American immigrants affected by HBV-related chronic active hepatitis. *Braz J Infect Dis*. 2007;11(3):311–313.

23. Akuta N, Suzuki F, Kawamura Y, et al. Virological response and hepatocarcinogenesis in lamivudine-resistant hepatitis B virus genotype C patients treated with lamivudine plus adefovir dipivoxil. *Intervirology.* 2008;51(6):385–393.

24. Chen P, Yu C, Wu W, et al. Serolological Profile Among HBsAg-Positive Infections in Southeast China: A Community-Based Study. *Hepat Mon*; 2013;13(1):e7604.

25. Akere A, Akande KO, Oke TO, et al. Hepatitis B Virus Infection: Characteristics of Patients, Frequency and Significance of Viral Markers. *J Hepat Res*. 2015;2(1):1021.

26. Kolou M, Katawa G, Salou M, et al. High Prevalence of Hepatitis B Virus Infection in the Age Range of 20-39 Years Old Individuals in Lome. *Open Virol J*. 2017;11:1–7.

27. Salih EY, Saeed WSE, Satti MMH, et al. Hepatitis B virus Genotypic Patterns in Sudan Reflects Population Movements. Glob J Virol Immunol. 2018;2(10):176–180.

28. Lee HW, Kim SU, Oidov B, et al. Comparison between chronic hepatitis B patients with untreated immune-tolerant phase vs. those with virological response by antivirals. *Sci Rep*. 2019;9:2508.

29. Ergunay K, Balaban Y, Cosgun E, et al. Epidemiologic trends in HBV infections at a reference centre in Turkey: an 11-year retrospective analysis. *Ann Hepatol*. 2012;11(5):672–678.

30. Lai MC, Tong MJ, Nowicki MJ, et al. Is anti-HBc IgM a useful clinical test in patients with HBsAg-positive chronic hepatitis or primary hepatocellular carcinoma? *Hepatol*. 1988;8(3):514–517.

31. Smith HM, Lau JY, Davies SE, et al. Significance of serum IgM anti-HBc in chronic hepatitis B virus infection. *J Med Virol*. 1992;36(1):16–20.

32. Kuhns MC, Kleinman SH, McNamara AL, et al. REDS Study Group. Lack of correlation between HBs Ag and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. *Transfusion*. 2004;44(9):1332–1339.

33. Bonino F, Piratvisuth T, Brunetto MR, et al. Diagnostic markers of chronic hepatitis B infection and disease. *Antivir Ther*. 2010;15(3):35–44.

34. Xiang Y, Chen P, Xia JR, et al. A large-scale analysis study on the clinical and viral characteristics of hepatitis B infection with concurrence of hepatitis B surface or E antigens and their corresponding antibodies. *Genet Mol Res*. 2017;16(1):gmr16019102.

35. Cheng J, Dai Y, Yan L, et al. Clinical Characteristics and Correlation Analysis of Subjects with Chronic Hepatitis B Virus (HBV) Infection and Sustained Low Levels of Hepatitis B Surface Antigen (HBsAg). *Med Sci Monit*. 2018;24:1826–1835.

36. Akbar SMF, Al-Mahtab M, Khan SI. Nature of Host Immunity during Hepatitis B Virus Infection and designing Immune Therapy. *Euroasian J Hepato-Gastroenterol*. 2018;8(1):42–46.

37. Ma Z, Zhang E, Gao S, et al. Toward a Functional Cure for Hepatitis B: The Rationale and Challenges for Therapeutic Targeting of the B Cell Immune Response. *Front Immunol*. 2019;10:2308.

38. Lada O, Benhamou Y, Poynard T, et al. Coexistence of Hepatitis B Surface Antigen (HBs Ag) and Anti-HBs Antibodies in Chronic Hepatitis B Virus Carriers: Influence of "a" Determinant Variants. *J Virol*. 2006;80(6):2968–2975.