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Hepatitis B virus infection in post-vaccination South Africa: Occult HBV infection and circulating surface gene variants

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ABSTRACT

Background and objective: The aim of this study was to investigate the prevalence of occult hepatitis B virus (HBV) infection and the HBV surface (S) gene variants circulating in the South African population after nearly two decades of universal hepatitis B vaccination.
Study design: From a previous serosurvey, 201 serum samples with serological evidence of exposure to HBV were identified and these were stratified into post- and pre-vaccine introduction populations. For all samples, HBV DNA was screened and quantified using a real-time PCR assay and results analysed together with HBV serological markers. Where HIV results were available, subset analysis was performed. The HBV S gene was PCR-amplified and sequences analysed for a total of 37 isolates.
Results: The prevalence of occult HBV infection reduced from 70.4% in the pre-vaccine introduction era to 66.0% post-vaccine introduction. There was an association between HIV infection and an increase in prevalence of occult HBV infection within the post-vaccine introduction population, although this was not statistically significant. Furthermore, sequence analysis revealed the following HBV subgenotypes; A1 ($n=34$), A2 ($n=2$) and a rare D4 isolate. HBV S gene variants, including diagnostic escape mutants were isolated.
Conclusion: There was a decline in the prevalence of occult HBV infection in post-vaccination South Africa, although the disease burden remains significant in the HIV co-infected population. After nearly two decades of a universal hepatitis B vaccination programme, no positive selection of vaccine escape mutants were observed.

1. Background

South Africa is hyper-endemic for hepatitis B virus (HBV) infection which is associated with fatal chronic sequelae such as liver cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC) [21,27]. Prior to hepatitis B vaccine introduction, the burden of hepatitis B in the country was chiefly driven by the high prevalence (>10%) of childhood (<5 years) infections accounting for ~3 million potentially fatal chronic HBV infections [19,44].

In keeping with the World Health Assembly's 1992 recommendation for controlling the hepatitis B burden in hyper-endemic regions, the hepatitis B vaccine was introduced into the South African Expanded Programme on Immunisation (EPI-SA) in April 1995 [37,47]. Subsequent studies have reported a decline in the prevalence of chronic HBV infection since the vaccine's introduction [2,35,36]. What threatens the benefits of vaccination is the emergence of HBV S gene variants due to immune selection pressure from the HBV vaccine [50]. These variants, which may exhibit immune, diagnostic and vaccine escape, can also be transmitted [6,9,49]. While selection of these variants is a very rare event,

Abbreviations: HBsAg, hepatitis B surface antigen; Anti-HBs, antibody to hepatitis B surface antigen; Anti-HBc, antibody to hepatitis B core antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus.

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vaccine escape mutants have been isolated in several countries with longstanding hepatitis B vaccination programmes, such as Taiwan [18,7] and The Gambia [12,46].

It is reasonable to expect the remarkable impact the hepatitis B vaccine has had on the prevalence of chronic HBV infection to be similar on occult HBV infection (OBI) which is also prevalent in South Africa, especially in the human immunodeficiency virus (HIV) infected population [28,25]. Clinically, OBI may be benign but is associated with increased risk of reactivation of HBV infection during immune compromise (for example during HIV infection) and can hasten progression of liver disease and hepatocarcinogenesis [38]. The public health concern is in the risk of transmission of HBV from asymptomatic and misdiagnosed occult HBV infected individuals to susceptible persons [30,38].

OBI is defined as the presence of HBV DNA in the hepatocytes or serum of individuals who test negative for the HBV surface antigen (HBsAg) by current available assays [33]. Various molecular mechanisms leading to the development of OBI have been described, this including mutations within the HBV S gene which may affect HBsAg detectability and result in an occult status [32,34].

2. Objective

The aim of this study was to examine the prevalence of OBI and investigate the HBV S gene variants circulating in a South African population post-vaccine introduction.

3. Study design

From a previous health facility-based hepatitis B serosurvey [2], 201 serum samples which tested positive for serological makers of exposure to HBV; HBsAg and/or antibody to HBV core antigen (anti-HBc), were identified out of a total sample size of 1206. These serologically exposed samples were the focus of this study. The serum samples were stratified by age based on the year (1995) the hepatitis B vaccine was introduced into EPI-SA, into a post-vaccine introduction population (POVP, 1–16 year olds) and a pre-vaccine introduction population (PRVP, 17–25 year olds). Where HIV results were available, a subset analysis of results was performed. The hepatitis B vaccination status of patients was not collected for the overarching study so as to mimic population-based nationwide hepatitis B serosurveys.

3.1. HBV DNA testing

Viral nucleic acid was extracted from 150 μl of each of the 201 serum samples using the Nucleospin® Viral Nucleic Acid Isolation kit (Macherey-Nagel, Düren, Germany). These extracts were screened for HBV DNA using an in-house quantitative real-time PCR (qPCR) assay (5X HOT FIREPol® EvaGreen® qPCR Mix Plus, Solis BioDyne, Estonia) performed on the LightCycler® 2.0 Real-Time PCR System (Roche, Germany). Primers designed to target the HBV basal core promoter (BCP) region (BcP1:5′-ACCACCAAATGCCCCTAT-3′ and BcP2:5′-TTCTGCGACGCGGCGA-3′) [17] were employed. A conversion factor of 2.8 copies/ml to IU/ml was used for all viral loads determined [31]. To avoid cross-contamination, physically separated rooms were used for HBV DNA extraction, qPCR master mix preparation and loading of the template. The possibility of false positives was excluded by performing conventional nested PCR on selected qPCR positive samples with varying viral loads (between 10^2 and 10^4 copies/ml) and analysing the sequenced amplicons.

4. HBV S gene amplification and sequencing

For amplification of HBV DNA in the S region, a conventional nested PCR assay was performed using two sets of primer pairs (first round: S1 = 5′-CCTGCTGGTGGCTCCAGTTC-3′, S2Na = 5′-CCACCATTCK(G/T)TTGACATACTTTCCA-3′ and second round: S6Bs = 5′-GATCCGAGGACTGGGGAC-3′, S7Ps = 5′-GGTTAGGGTTTAAATGTATAC-3′) in a two-step reaction for all HBV DNA positive samples with viral loads >35.7 IU/ml (detection limit by this PCR assay). The cycling conditions used for amplification of the S gene were as outlined previously by Mphahlele et al. [28]. Confirmation of the desired amplicon (~681 bp) was performed by gel electrophoresis on a 2% agarose gel. Direct sequencing was then carried out with the ABI3500XL Genetic Analyzer (Applied Biosystems, CA, USA), using primers S6Bs and S7Ps.

4.1. Phylogenetic analysis of HBV S gene sequences

All sequences were edited and aligned using ChromasPro 1.49Beta (Technelysium Pty. Ltd.) and BioEdit Sequence Alignment Editor [15] respectively, and then analysed for both nucleotide and amino acid variations. For phylogenetic analysis, representative sequences from different HBV subgenotypes were retrieved from the GenBank database for comparison with study sequences. All alignments were performed using the neighbour-joining method implemented in Clustal X [41]. The statistical robustness and reliability of the branching order was confirmed by bootstrap analysis using 1000 replicates [10]. Phylogenetic inference was also performed using a Bayesian Markov Chain Monte Carlo (MCMC) approach as implemented in the BEAST v1.8.0 program [8].

5. Results

5.1. Sample population

Serum samples ($N = 201$) were collected from various health facilities from five of the nine provinces in South Africa; Gauteng (49.8% [$n = 100$]), North West (40.3% [$n = 81$]), Mpumalanga (6.0% [$n = 12$]), Limpopo (3.5% [$n = 7$]), and the Northern Cape (0.5% [$n = 1$]). Of the 201 serum samples, 62 (30.8%) were classified by age into the POVP and 139 (69.2%) into the PRVP based on national introduction of the vaccine in 1995 (Table 1). The demographic background of the sample population has been described previously [2].

5.2. Prevalence of OBI

While 17.3% (24/139) and 14.5% (9/62) of the PRVP and POVP respectively were HBsAg positive, the majority of both populations were HBsAg-negative (Table 1). This proportion of HBsAg negative samples served as the population for evaluation of the prevalence of OBI. HBV DNA was detectable in 70.4% (81/115) and 66.0% (35/53) of the HBsAg negative PRVP and POVP respectively, with mean viral loads of 336.42 IU/ml and 356.07 IU/ml. Of the populations with OBI, 37.0% (30/81) of the PRVP and 40.0% (14/35) of the POVP had the isolated anti-HBc serological profile (Table 1).

Overall, HIV results were available for 34/139 (24.5%) and 23/62 (37.1%) of the PRVP and POVP respectively, with 70.6% (24/34) and 56.5% (13/23) being HIV positive. There was an association between HIV infection and an increase in the prevalence of OBI within the POVP (91.7% [11/12] in the HIV positive versus 70.0% [7/10] in the HIV negative subsets) although this was not statistically significant (Table 2).