Increased Detection of HBV DNA and Mutational Analysis in HBsAg Negative Specimens at NHLS/DGM.

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BACKGROUND

The standard approach to HBV diagnosis is hepatitis B surface antigen (HBsAg) screening[1]. However, the rising detection of HBV DNA in HBsAg negative specimens indicates a diagnostic gap. The underdiagnosis of HBV due to reliance on HBsAg screening poses a significant public health risk, as individuals with undetected HBV infection unknowingly transmit the virus [2,3,4]. Moreover, these individuals remain at risk of liver disease progression without appropriate intervention[4]. Addressing this diagnostic gap is critical for improving patient outcomes, enhancing the accuracy of HBV surveillance, and reducing the overall disease burden associated with HBV. The aim of this study was to investigate HBV DNA and mutations in HBsAg negative specimens at Dr George Mukhari National Health Laboratory Services (NHLS/DGM).

METHODS

This descriptive study used 444 stored residual serum samples that tested negative for HBsAg during routine diagnosis, from March 2021 to May 2022 at National Health Laboratory Services, Dr George Mukhari (NHLS/DGM).

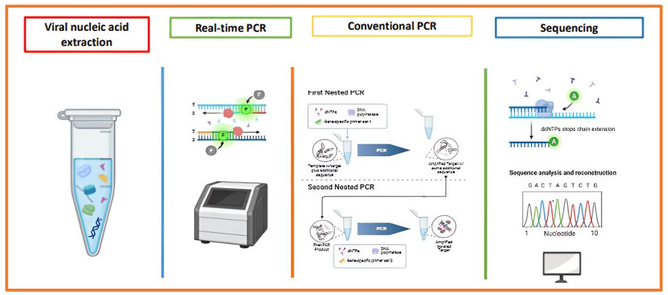


Figure 1: Overview of laboratory methods for viral detection and analysis. HBV DNA was extracted using the High pure viral nucleic extraction kit following the manufacturers instructions.HBV-DNA was detected using a qrt-PCR assay (detection limit: 45 IU/ml), followed by conventional in-house nested PCR to amplify the S gene. Positive and negative controls were included in each run. Positive samples underwent sequencing (Inqaba Biotech, South Africa) for mutational and phylogenetic analysis. Sequence analysis was performed using bioinformatics tools: Chromas Pro (2.0) for manual editing, BioEdit (7.0) for alignment and MEGA (11.1.13) for phylogenetic analysis.

KEY MESSAGES

HBV DNA detection in HBsAg-negative specimens: suggests that HBsAg-based screening alone may not be sufficient for HBV diagnosis.

Low viral load was detected in many specimens: Among the HBV DNA positive specimens, 41% had viral loads below 200 IU/mL, indicating potential occult HBV infection (OBI).

Higher HBV DNA detection in younger adults and HIV positive Individuals: The most affected age group was 20-29 years, and HIV positive specimens had a higher prevalence of HBV DNA.

Recommendation for revised diagnostic criteria: The findings highlight the need to reconsider HBV diagnostic methods, advocating for alternative assays that are as cost-effective as HBsAg screening but offer improved sensitivity.

RESULTS

Most of the specimens tested were from females **(65%)** with a median age of 34. Sixty-nine **(16%)** samples were HBV DNA positive with a median viral load of 289 IU/mL. Of the 69 samples, 28 **(41%)** had viral loads below 200 IU/mL, indicating true OBI, whereas the remaining 41 **(59%)** had viral loads above 200 IU/mL, suggesting false-OBI (Table 1). Most affected age group was 20-29 (33%), and HIV positive group had more HBV DNA than HIV negative group.

Table1: Sociodemographic and laboratory characteristics of study patients according to HBV DNA status.

	HBV DNA Status		
Characteristics	**Overall N=444**	**Negative N=375**	**Positive N=69**
Age[1] (years; IQR: 25%-75%)	34 [24- 42]	34 [32-35]	39 [26-50]
(Missing)	61	49	12
Age Interval			
< 20	9% (35 / 383)	10% (32 / 326)	5% (3 / 57)
20 - 29	33% (127 / 383)	34% (111 / 326)	28% (16 / 57)
30 - 39	26% (98 / 383)	27% (87 / 326)	19% (11 / 57)
40 - 49	17% (65 / 383)	17% (54 / 326)	19% (11 / 57)
50 <	15% (58 / 383)	13% (42 / 326)	28% (16 / 57)
(Missing)	61	49	12
Sex			
Female	65% (287 / 440)	66% (248 / 375)	57% (39 / 68)
Male	35% (153 / 440)	33% (124 / 375)	43% (29 / 68)
(Missing)	4	3	1
HBsAg titre[1] (Sco; IQR: 25%-75%)	0.30 [0.26-0.47]	0.30 [0.26-0.46]	0.38 [0.25-0.49]
HIV status			
Negative	8% (17 / 203)	7% (13 / 176)	13% (4 / 30)
Positive	92% (186 / 203)	93% (163 / 176)	87% (26 / 30)
(Missing)	241	203	39
HBV DNA viral loa UI/mL	290 [99-789]	0	290 [99-789]

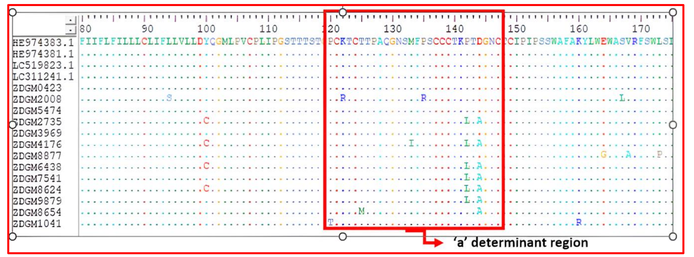


Figure 2: Surface region mutational analysis of sequences showing the 'a' determinant. The mutations T125M, M122I, P135R, P142L and D144A were identified in 52% of the specimens. Mutations in this region play an important role in diagnosis and immune development in HBV infection.

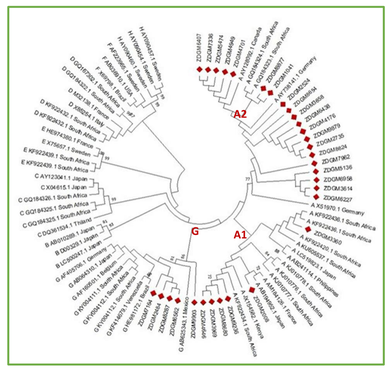


Figure 3: A rooted phylogenetic tree showing the relationship between the isolated HBV genome sequences with 35 reference sequences. The neighbour joining tree was constructed using MEGA version 11.0.13 with 1000 bootstrapping replicates. Bootstrap values greater than 70% are shown on the branch nodes. Sequences from this study are denoted as "ZDGM" and the red dot, while reference sequence have an accession number and country for genotypes/sub genotypes as presented on Genbank. Genotyping identified five sequences as genotype A1, 21 genotype A2 and five genotype G.

CONCLUSIONS

In conclusion, the study found a relatively high HBV DNA prevalence in HBsAg negative samples. Furthermore, this prevalence highlights the need for improved screening and confirmatory diagnostic tests for HBV that can detect variants (escape and occult related mutations) and low viral loads. This study demonstrated the need of validating other HBV markers for accurate diagnosis of HBsAg negative infection, as relying solely on HBsAg screening assays may overlook cases of HBV infection. As such this can lead to potential misdiagnosis, underdiagnosing and missed opportunities for appropriate intervention and monitoring.

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