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SHORT COMMUNICATION

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Characterization of hepatitis delta virus strains spreading in Abuja, Nigeria

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Abstract

Hepatitis delta virus (HDV) is responsible for the most severe form of liver disease in humans. So far, eight genotypes (HDV-1 to -8) have been individualized worldwide. Little is known about HDV strains that spread in Nigeria. HDV genotyping was performed in 15 anti–HDV positive samples from a cohort of 306 hepatitis B virus (HBV)-infected patients in Abuja (Nigeria). Phylogenetic analyses revealed 90% were HDV-1, two among them clustering with European/Asian HDV-1, the remaining one being HDV-6. It was also found that two members of a couple superinfected with the same HDV strain, were enveloped by two different HBV strains of genotype E.

KEYWORDS

blood, epidemiology, hepatitis B virus, hepatitis D virus, satellite, subviral agents, virus classification

1 | INTRODUCTION

Hepatitis delta virus (HDV) is a satellite of hepatitis B virus (HBV), that requires the HBV envelope (HBV surface antigen or HBsAg) for its assembly and propagation.[1] HDV is the sole member of delta virus genus and is thought to be responsible for the most severe acute and chronic liver disease in humans.[2] Global estimates of chronic HBsAg carriage ranges from 248 to 364 million[3-5] and approximately 5% to 20% of these are co- or superinfected with HDV.[6]

The HDV genome is a 1.7-kb, circular, single-stranded RNA of negative polarity. An open reading frame, situated on the "antigenome" (a replication intermediate, the exact complement of the genome) encodes the two isoforms of the hepatitis Delta antigen (HDAg): the small (S-HDAg), and the large hepatitis delta antigen (L-HDAg) of, respectively, 24 and 27 kDa, and 195 and 214 amino acid content. This is due to an RNA editing step involving the host adenosine deaminase acting on double-stranded RNA-1 (ADAR 1), which converts the UAG (Stop codon) of the S-HDAg to a Tryptophan codon (UGG) allowing the translation of the downstream amino acid of the S-HDAg.[7]

HDV genus is characterized by a very high genetic diversity. Eight genotypes (HDV-1 to -8) have been individualized across the world, showing a characteristic geographical distribution.[8] HDV-1 is widely distributed, very likely from an ancestral African strain, which has colonized the rest of the world.[8] HDV-2 and -4 are found in Eastern and Northern Asia; HDV-3 is restricted to South America, while HDV-5 to -8 are majorly spreading in Sub-Saharan Western and Central Africa and in countries of African migrations. Of note one HDV-8 strain has also been isolated in Brazil, very likely related to ancient migration related to the slave trade during the 16th to the

TABLE 1 Characteristics of the study population

Sample ID	Age/sex	HDV-Ab	HDV-VL (LogUI/mL)	HDV genotype	HBV genotype	HIV status
NGR-D-005	33/M	POS	ND	N/D	N/D	POS
NGR-D-006	30/F	NEG	N/A	N/A	N/D	NEG
NGR-D-008	24/F	POS	5.97	1	N/D	NEG
NGR-D-010	31/M	POS	ND	N/D	N/D	POS
NGR-D-011	50/M	NEG	N/A	N/A	N/D	NEG
NGR-D-012	30/M	POS	ND	ND	N/D	NEG
NGR-D-020	31/M	POS	ND	ND	N/D	POS
NGR-D-14	**32/M**	**POS**	**6.06**	**1**	**E**	**NEG**
NGR-D-140	18/M	POS	ND	ND	N/D	POS
NGR-D-15	**29/F**	**POS**	**7.67**	**1**	**E**	**NEG**
NGR-D-16	33/M	NEG	N/A	N/A	N/D	NEG
NGR-D-17	23/M	POS	5.37	1	N/D	NEG
NGR-D-21115	41/M	POS	3.99	1	N/D	NEG
NGR-D-21A	23/F	POS	D	1	E	POS
NGR-D-2A	21/F	POS	1.72	1	N/D	NEG
NGR-D-3B	32/M	POS	1.37	6	N/D	POS
NGR-D-7A	32/F	POS	7.41	1	N/D	POS
NGR-D-N04	18/F	POS	D	1	N/D	NEG
NGR-D-018	37/F	INSUFF	N/A	N/A	N/A	POS

Note: Samples from members of a couple are given in bold.
Abbreviations: D, detectable but unquantifiable (<1000 IU/mL); F, female; HBV, hepatitis B virus; HDV, hepatitis delta virus; HDV-Ab, HDV antibody; HDV-VL, HDV viral load quantification; HIV, human immunodeficiency virus; INSUFF, insufficient sample; M, male; N/A, not applicable; ND, not detectable.

19th centuries.[9] The consequences of such a large molecular diversity on the severity of the hepatic disease remain to be fully explored and understood. Previous studies claimed that the course of the disease might be influenced by genotypes: HDV-1 being associated with more severe disease than HDV-2 and-4[10] and HDV-3 associated with a particularly aggressive evolution with more fulminant hepatitis and deaths.[11] More recent studies and personal observations from Roulot and Gordien[12] showed that the evolution toward cirrhosis was significantly higher in patients infected with European HDV-1 than with African HDV-1, and within African patients, those infected with HDV-5 than with HDV-1.

Therefore, taking advantage of over 300 HBV-infected plasma samples archived in the repository which found 15 confirmed positive anti–HDV-antibody samples, the genetic variability of HDV strains that spread in the Federal Capital Territory, Abuja Nigeria was performed.

2 | METHODS

2.1 | Study samples/participants

Nineteen anti–HDV (HDV-Ab) positive plasma samples found during a previous screen[13] were analyzed in this study. These samples were collected between April and October 2016 from two cohorts, 1 of 113 HBV/human immunodeficiency virus (HIV) coinfected individuals attending antiretroviral (ARV) clinics and the other of 193 HBsAg-positive, HIV-negative blood donors from blood donation units of four selected hospitals in the Federal Capital Territory (FCT), Abuja Nigeria. Ethical approval for the study was obtained from FCT Health and Research Board (FHREC/2016/01/24/06-04-16)

2.2 | HDV antibody screening

Eighteen out of the 19 samples had enough volume to be retested for total anti-HDV, using the commercial ETI-AB-DELTAK-2 kit (DiaSorin, Saluggia, Italy). The 15 confirmed positive samples according to manufacturer's instructions were further explored for both HDV-RNA viral load and HBV and HDV genotyping.

2.3 | HDV and HBV genotyping

2.3.1 | Nucleic acid extraction

HDV RNA and HBV DNA were extracted from 250 µL of plasma using the QIAamp DSP Virus Spin Kit MiniElute virus vacuum kit (QIAGEN, Courtaboeuf, France) and then eluted in 30 µL of RNAse free water according to manufacturer's instructions.

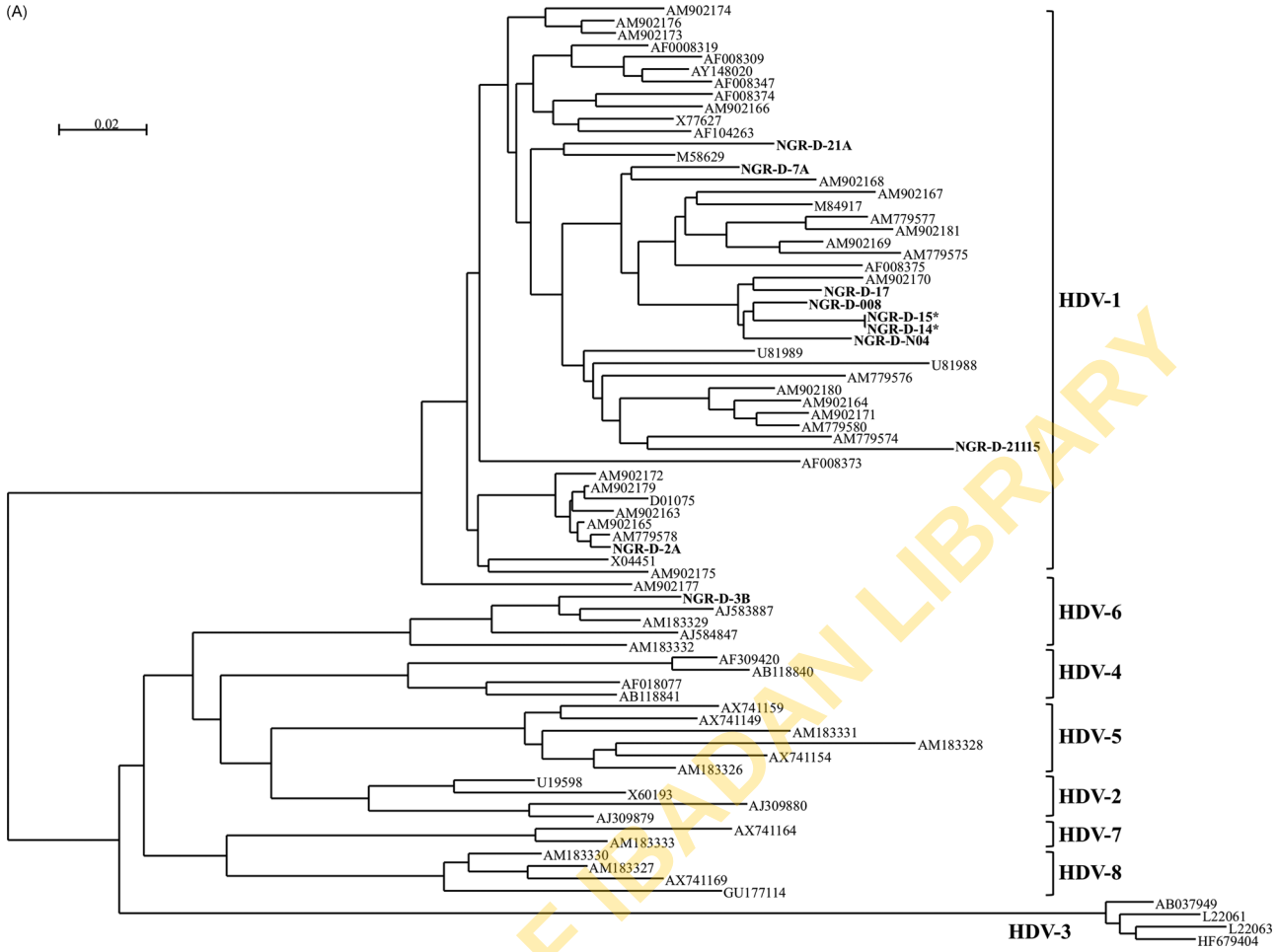

FIGURE 1 A, Phylogenetic analysis of study hepatitis delta virus (HDV) Nigerian isolates. B, Phylogenetic tree showing in bold the characterized hepatitis B virus (HBV) Nigerian isolates. * Isolates recovered from the members of the couple infected with the same HDV strain

HDV *R0* amplicons were obtained by amplification by reverse-transcription polymerase chain reaction (RT-PCR) of the so-called *R0* region of HDV genome (400-bp long), encompassing nucleotide 920 to 1289 exactly as described earlier.[8]

HBV Pre S1 amplicons were obtained by amplification by PCR of a region of the pre S1 gene (479-bp long) that spans nucleotides 2817 to 80 as described earlier[14] particularly for two samples of interest NGRD014 and NGRD015 isolated from a couple infected a priori by the same HDV strains. HBV genotype was obtained by an HBV hemi-nested PCR as reported in 2012.[14] Briefly, the entire HBV genome was amplified first using P1 and P2 primers described previously by Gunther et al[15] followed by nested PCR described elsewhere.[14]

After purification of the amplicons using a Microcon-PCR 50 column filter (Millipore, Molsheim, France), sequences were determined on the ABI PRISM 3100 Analyzer (PE Applied Biosystems, Waltham, MA) using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

2.3.2 | Phylogenetic analyses

The HDV and HBV sequences obtained were compared with reference sequences retrieved from Genbank. Alignments were carried out after manual minor modifications, using the CLUSTAL W program in MEGA 5 software with default settings.[16] Phylogenetic trees were constructed by the neighbor-joining method using MEGA 5 software with the Kimura-2 parameter model. To confirm the reliability of phylogenetic tree topologies, bootstrap reconstruction was carried out 1000 times.

2.3.3 | Nucleotide accession number

The *R0* and HBV pre S1 sequences characterized in this study are under submission in GenBank.

2.4 | HDV-RNA viral load quantification

HDV viral load quantification (HDV-VL) was performed as described earlier using the Eurobioplex HDV kit.[17]

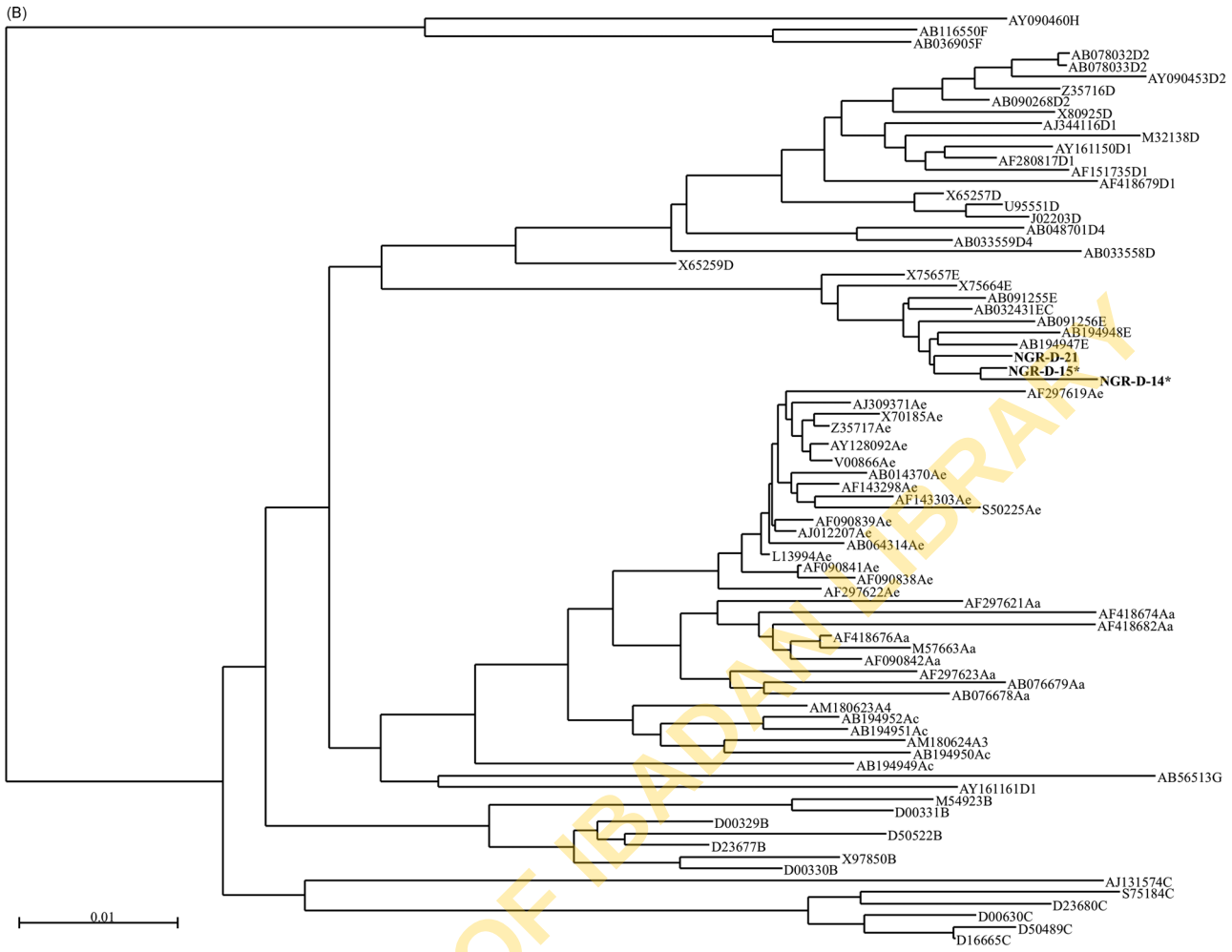

FIGURE 1 Continued

3 | RESULTS AND DISCUSSION

Table 1 summarizes the main characteristics of the cohort of patients. The median age was 38 years (range: 18-50). Of the 18 available samples considered as HDV-Ab positive, 15 (83.3%) were confirmed using another ELISA commercial assay known for its high sensitivity and specificity. The three remaining samples were borderline or weekly positive during the initial screen[13] as a consequence we concluded they were likely false positives. Of note, 7 of these 15 confirmed anti–HDV-Ab-positive samples (46.7%) were also coinfected with the HIV indicating very likely the same route of infection. This study confirms that HDV seroprevalence in Nigeria is low, about 4.9% (15 of 306) of HBsAg-positive patients, as reported elsewhere;[18] however, this rate may be an underestimation, as suggested in the large meta-analysis conducted by Stockdale et al,[19] which found 7.1% among the general population in West Africa. HDV RNA was detected in 10 (66.7%) of the 15 HDV-Ab confirmed samples thus indicating an active infection in these individuals. One strain belonged to genotype HDV-6 and segregated with strains from Nigeria, neighboring Cameroon and other West African countries. The nine remaining were HDV-1. HDV-1 and HDV-5 to -8 were described in Africa; however, HDV-7 and -8 have not yet been characterized in Nigeria.[20] On the phylogenetic tree (Figure 1A), seven Nigerian strains clustered with other sub-Saharan African-HDV-1 strains, and the remaining two, NGR-D-2A and NGR-D-21A, with European/Asian HDV-1. As described elsewhere[14] they exhibited, respectively, a Serine and an Alanine (specific markers of African and European/Asian HDV-1 strains) at position 202 of the large delta protein (data not shown). Remarkably, two HDV strains, NGR-D-14 and NGR-D-15, isolated from two members of a couple, seem to be the same infected strain. However, the HBV strains from the two individuals (Figure 1B), while belonging to the same genotype E, were different. It, therefore, seems that the two members of this couple have very likely been first infected independently by two different HBV/E-strains, then subsequently superinfected by the same HDV strain (NGR-D-14/NGR-D-15).

In summary, HDV prevalence seems to be low in Nigeria (around 5%). Studies in other parts of the country are needed to have the true

reality of HDV spreading. Most strains (70%) belong to the African-HDV-1 group, while two European /Asian HDV-1 were found, and there is evidence that HDV-6 and very likely other specific African genotypes might also be circulating in the Country.

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