Journal of Clinical Virology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Molecular epidemiology of hepatitis delta virus in the Western Pacific region

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ARTICLE INFO

Article history:
Received 12 March 2014
Received in revised form 22 May 2014
Accepted 30 May 2014

Keywords:
Hepatitis delta virus
Molecular epidemiology
Western Pacific region
Oceania
Genotype
African origins

ABSTRACT

Background: Hepatitis delta virus (HDV) is a defective RNA virus requiring the presence of the hepatitis B virus (HBV) for the completion of its life cycle. Active replication of HDV can lead to severe hepatitis, and although present worldwide has an irregular geographical distribution, especially in the Asian Pacific region.
Objectives: The aim of this study was to determine the prevalence and molecular epidemiology of HDV isolates in Oceania following the 1998 evaluation of the hepatitis B vaccine program.
Study design: Sera collected from 184 hepatitis B surface antigen (HBsAg) positive Pacific Islanders living in Micronesia, Polynesia and Melanesia were tested for HDV RNA.
Results: Twenty of 54 patients with chronic hepatitis B (CHB) from Kiribati were positive for serum HDV RNA (37%), whilst sera from patients with CHB from Tonga (59), Fiji (42) and Vanuatu (29) were negative. The mean HDV RNA load for the 20 samples was 7.00 $\log_{10}$ copies/mL. Phylogenetic analysis revealed that the Kiribati HDV isolates were of genotype 1 and clustered with a previously published isolate from Nauru forming a distinct clade of Pacific HDV. All Micronesian isolates contained a serine at codon 202 of large hepatitis delta antigen (L-HDAg) demonstrating possible relatedness to strains of HDV-1 of African origin.
Conclusions: This study has confirmed endemic HDV infection in Micronesia and identified Kiribati as having amongst the highest prevalence for HDV viraemia in patients with CHB. Further investigations are ongoing into the origins of this unique HDV Pacific strain, and its inter-relationship with HBV.

1. Background

Infection with hepatitis delta virus (HDV) occurs in individuals by either super-infection in someone already infected with the hepatitis B virus (HBV) or by co-infection at the time of HBV transmission. Serological surveys have shown that infection with HDV is present worldwide [1] but has an irregular distribution, being endemic in the Mediterranean basin, parts of Africa, the Middle East and South America [2,3]. The prevalence in Western Europe [4], Scandinavia [5] and Australia [6] is low and largely confined to injecting drug users.

Hepatitis B infection is endemic in many communities in the Western Pacific region [7], with carrier rates amongst the highest in the world [8–10]. The prevalence of HBsAg positivity amongst Pacific Islanders ranges from 7% to over 30% of the adult population [11]. However, an astounding 98% of the population in the Republic of Kiribati have been found to carry markers of HBV infection, and 32% were shown to be HBsAg positive [12]. In contrast, the distribution of HDV infections defined by serological testing in the Western Pacific region is highly irregular. The major foci of infections have been found in Nauru, Kiribati, Niue and Western Samoa, whilst it is extremely low or completely absent in the Cook Islands, Tahiti, New Caledonia, Vanuatu, New Zealand and Fiji [7,13]. Nonetheless, akin to hepatitis B prevalence, the highest prevalence of anti-delta (anti-HD) has been found in Kiribati with between 60% and 80% of HBV infected individuals anti-HD positive [13,14].

Abbreviations: HBV, hepatitis B virus; HDV, hepatitis delta/D virus; HDAg, hepatitis delta antigen; L-HDAg, large hepatitis delta antigen; HBsAg, hepatitis B surface antigen.

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http://dx.doi.org/10.1016/j.jcv.2014.05.021
1386-6532/

The hepatitis delta virion is a chimaera composed of the HDV RNA genome with its associated hepatitis delta antigen (HDAg), both of which are enveloped by the HBsAg of HBV. The HDV genome consists of a circular, rod-shaped, minus strand RNA [15]. Initial molecular epidemiological analysis of HDV RNA sequences revealed three major genotypes with different geographic distributions [16]. Genotype 1 is predominant in the United States, Europe, Africa and the Middle East; genotype 2 has been isolated in Japan, Taiwan and Russia [17]; whilst genotype 3 is the only genotype isolated in the north of South America, where HBV genotype F predominates [18,19]. In Taiwan and Japan, a fourth genotype, has been identified [20] and recent sequence analysis of HDV isolated from sera of African origin has led to characterisation of genotypes 5, 6, 7 and 8 [21]. The HDV genotypes can differ by 30–40% of the genome sequence [3,22], with highly conserved domains located around the genomic and anti-genomic RNA ribozyme and auto catalytic cleavage sites and the RNA-binding domain of HDAg [23,24]. Furthermore, HDV genotype 1 isolates related to strains of African origin are more likely to have a serine residue at codon 202 of the large HDAg rather than an alanine which has been suggested to constitute a marker of human migration [25].

There have been no previous studies of the molecular epidemiology of HDV infections in the Western Pacific region. In 1998, a study was performed to evaluate the efficacy of the infant hepatitis B immunisation programs in Fiji, Kiribati, Tonga and Vanuatu [11]. The samples collected provided the basis for further virological and molecular epidemiological studies on the Pacific strains of HBV [26–28] and HDV.

2. Objectives

The objective of this study was to define the molecular epidemiology of HDV isolated from individuals who reside in the Western Pacific, providing a reference point for future epidemiological comparisons and investigations.

3. Study design

3.1. Serum samples

HBsAg-positive samples were collected from four Pacific Islands shown in Fig. 1. A total of 184 serum samples were analysed: 54 from Kiribati, 29 from Vanuatu, 42 from Fiji, and 59 from Tonga. The demographic details of these individuals have been previously reported [11]. Written approval for all the initial and current survey procedures was obtained from the Department of Health in each country and informed consent was obtained from each adult or the mother or guardian of each participant [11].

3.2. Nucleic acid extraction and reverse transcription

HDV RNA was extracted from serum samples with the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) following the supplied instructions. The RNA was heated at 65 °C, and cooled on ice. Reverse transcription of RNA was performed using the SuperScript® VILO™ cDNA Synthesis Kit (Life Technologies, CA, USA), according to the manufacturer's instructions. HBV DNA was extracted from 200 μl of serum using the QIAamp DNA Mini Kit (QIAGEN) as per the manufacturer's instructions.

3.3. HDV screening PCR assay and sequencing

A 420 base pair (bp) product including the 3′ terminal end of the HDAg coding region was amplified using the primer set: HDV-01 (5′-GATGCCATGCCGACCCGAAGAGG-3′, nt 885–907) and HDV-01a (5′-AGAGGCAGGATCACCGACGAAGG-3′, nt 1305–1283) (sequence numbering according to Wang et al. [29]). Four microliters of cDNA was amplified in a 50 μl reaction mix containing 2 U HotStarTaq*Plus*DNA polymerase (QIAGEN), 0.5 mM dNTPs and 0.1 μM each primer. The thermocycling conditions were: 95 °C for 5 min, 50 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 10 min. The amplicons were purified with ExoSAP-IT (Affymetrix, CA, USA), and directly sequenced with the Big-Dye Terminator (Applied Biosystems, CA, USA) as previously described [30].

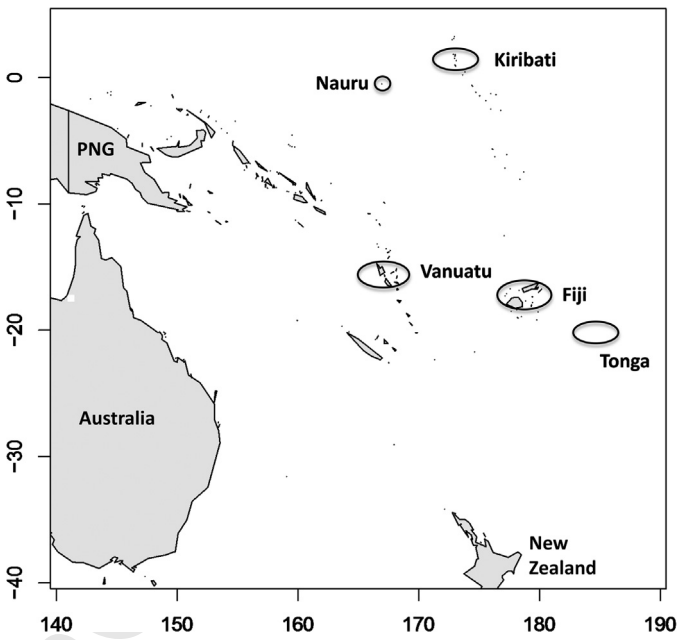

Fig. 1. Map of the Western Pacific region indicating the location of the four islands from which samples were obtained for this study, and Nauru.

3.4. Real-time quantitative PCR

All HBsAg positive serum samples were re-tested with a quantitative assay performed on the LightCycler (Roche Diagnostics, Mannheim, Germany) in order to determine the HDV RNA load using primers described by Schaper et al. [31]. Plasmid pDL445 containing an 843 bp fragment of the HDV genome and known copy number was used as a standard [32].

3.5. HDV genotyping and nucleotide divergence analysis

The amplicon of the routine screening PCR assay encompasses a 333 bp region of the 3′-end of the large delta protein (L-HDAg) coding sequence corresponding to nt positions 936–1269 [29,33]. The Kiribati HDV sequences were aligned with 68 HDV sequences from GenBank [34] using the MAFFT program [35], followed by manual correction using BioEdit v7.0.5 [36]. Phylogenetic and evolutionary divergence analyses were performed using the Molecular Evolutionary Genetics Analysis v5 software (MEGA 5) [37]. The Kiribati HDV isolates were genotyped based on the maximum likelihood (ML) phylogenetic tree generated using the GTR + G + I substitution model, as proposed by the model testing program in MEGA5, with 1000 bootstrap replicates. Evolutionary divergence analysis of the Kiribati isolates was performed using the maximum composite likelihood substitution model with 1000 bootstrap replicates.

Please cite this article in press as: Han M, et al. Molecular epidemiology of hepatitis delta virus in the Western Pacific region. J Clin Virol (2014), http://dx.doi.org/10.1016/j.jcv.2014.05.021