Virus Research 224 (2016) 6–11

Contents lists available at ScienceDirect

Virus Research

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

The hepatitis delta genotype 8 in Northeast Brazil: The North Atlantic slave trade as the potential route for infection

Max Diego Cruz Santos[a,*,1], Michele Soares Gomes-Gouvêa[a], Jomar Diogo Costa Nunes[b], Lena Maria Fonseca Barros[b], Flair José Carrilho[a], Adalgisa de Sousa Paiva Ferreira[b], João Renato Rebello Pinho[a,c,1]

[a] Laboratory of Tropical Gastroenterologyand Hepatology "João Alves de Queiroz and Castorina Bittencourt Alves", LIM-07, São Paulo Institute of Tropical Medicine and Department of Gastroenterology, School of Medicine, University of São Paulo, São Paulo, SP, Brazil
[b] Center of Clinical Research, University Hospital of Federal University of Maranhão, São Luís, Maranhão, Brazil
[c] Hospital Israelita Albert Einstein, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form 1 August 2016
Accepted 3 August 2016
Available online 8 August 2016

Keywords:
HDV-8
Slave trade
Maranhão
Brazil

ABSTRACT

Hepatitis Delta virus (HDV) is not well known, even though HDV and Hepatitis B virus (HBV) co-infection leads to severe forms of acute and chronic liver diseases. HDV is endemic in the Western Amazon region. Recently, the HDV genotype 8 was found in chronic patients followed at the center for liver studies in the Northeast Brazil, Maranhão. Previous studies suggested that this genotype was introduced in Maranhão during the slave trade. The presence of HDV in that study, which was done outside the Amazon region, led us to investigate whether the virus is found infecting individuals in other regions of Maranhão as well. Thus, we screened ninety-two HBsAg positive individuals from five Municipalities of Maranhão for anti-HD antibody and eight were found positive (8.7%). These eight positive individuals were submitted to polymerase chain reaction (PCR) to investigate active HDV infection. Half of them were positive for a fragment sequence of the delta antigen; their sequence samples were submitted to genotype characterization by phylogenetic analysis. All sequences clustered in a unique branch of the tree separated from the other branch described in Africa. Our study confirmed the presence of HDV-8 in Maranhão. These infected individuals had no evidence of contact with African people. Furthermore, we found individuals infected with HDV-8 in two more different municipalities. More studies like ours are urgent because the co-infection HBV/HDV is more difficult to treat. Identification of the endemic regions and implementation of healthy policies for preventing this infection are urgent in this region.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Hepatitis Delta virus (HDV) is not well known like other hepatotrophic viruses such as Hepatitis B (HBV) and C (HCV). HDV antigen and antibody were discovered less than four decades ago when a group of physicians investigated serum and liver biopsy of positive HBsAg patients (Rizzetto et al., 1977). HDV is the smallest virion to infect animal cells. The virus has a circular genome of around 1700 base pairs that includes a ribozyme that plays an import role in HDV replication (Alves et al., 2013; Rizzetto and Alavian, 2013; Taylor, 2014). This virus infects liver cells but differently than other known hepatotrophic viruses, it requires the surface antigen (HBsAg) of the HBV in order to enter liver cells and secrete new virions (Sureau, 2006; Taylor, 2014).

HDV is often found in the Mediterranean, Central Africa and Northern parts of South America (Radjef et al., 2004). In the Amazon region of Brazil, HDV, together with HBV, are a major public health burden (Bensabath et al., 1987; Braga et al., 2012). Furthermore, both HDV and HBV have a huge genomic diversion and these viruses split in groups named genotypes (and subgenotypes, for HBV), that show a characteristic geographic distribution (Deny, 2006; Kramvis, 2014). Currently eight genotypes of HDV were described, HDV-1 to HDV-8, with the exception of HDV-1, all genotypes are found in distinct geographic regions (Deny, 2006; Le Gal et al., 2012; Radjef et al., 2004). HDV-2 and 4 are found in the East Asia (Imazeki et al., 1990; Ivaniushina et al., 2001; Sakugawa et al., 1999); the HDV-3 is mainly described in the Amazon Basin (Crispim

* Corresponding author at: Laboratório de Gastroenterologia e Hepatologia Tropical, Instituto de Medicina Tropical, FMUSP, Av. Dr. Enéas Carvalho de Aguiar,500, 05403-000 São Paulo, SP, Brazil.
E-mail address: maxdiegocs@usp.br (M.D.C. Santos).
[1] Bolsista do CNpq, Brazil.

http://dx.doi.org/10.1016/j.virusres.2016.08.003
0168-1702/© 2016 Elsevier B.V. All rights reserved.

et al., 2014; Gomes-Gouvea et al., 2008; Gomes-Gouvea et al., 2009) and the HDV-5 to HDV-8 were found mainly in African individuals (Deny, 2006; Francois-Souquiere et al., 2016; Le Gal et al., 2012; Radjef et al., 2004).

Although in Brazil HDV is endemic in the Amazon region, mainly in the Western region, recently some cases were found outside this region, in the Maranhão state, Northeast Brazil (Barros et al., 2011). Until now only HDV genotype 3, in addition of few cases of HDV-1, has been identified in Amazon region (Crispim et al., 2014; Gomes-Gouvea et al., 2008; Gomes-Gouvea et al., 2009; Viana et al., 2005), whereas in Maranhão state the African genotype HDV-8 was found infecting Brazilian individuals, which led the authors to suggest that this genotype was introduced in the region during the slave trade (Barros et al., 2011). The history of North Atlantic slave trade in the state of Maranhão corroborates with the same hypothesis of the introduction of the HDV genotype 8 through the slave trade (Silva, 2008).

The presence of HDV in Maranhão, and outside the Amazon region, led us to investigate whether the virus are spread in other municipalities of Maranhão besides those already studied previously (Barros et al., 2011), in order to certify if these findings were not only isolated cases as already found in some regions of Brazil (Mendes-Correa et al., 2011; Strauss et al., 1987). Thus, herein we describe the second report of genotype 8 of HDV in the state of Maranhão, Brazil.

2. Material and methods

2.1. Samples and ethical approval

Ninety-two individuals who were positive for HBsAg serological marker among 3860 individuals, from five municipalities in Northeastern Maranhão, participated in this study (Fig. 1). The research ethnics committee of University Hospital, Federal University of Maranhão (HUUFMA) approved this study and written informed consent was obtained from all individuals that agreed to participate. We screened the 92 samples for anti-HD using the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (DiaSorin, Italy).

2.2. HDV RNA and HBV DNA amplification and sequencing

HDV RNA and HBV DNA were extracted using the QIAamp MiniElute kit (Qiagen®, Hilden, Germany). Fragments of 403 base pairs (bp) for HDV (partial delta antigen genomic region) and 1306 bp for HBV (partial DNA polymerase- and surface antigen-encoding genes) were amplified by nested PCR (polymerase chain reaction) according to procedures described elsewhere (Gomes-Gouvea et al., 2015; Gomes-Gouvea et al., 2008). PCR fragments were purified using ChargeSwitch® PCR Clean-Up Kit (Life Technologies, USA). Sequencing procedures are described in a previous study (Gomes-Gouvea et al., 2015; Gomes-Gouvea et al., 2008). We performed carefully all procedures to avoid contamination or false-positive results (Kwok and Higuchi, 1989).

2.3. HDV and HBV genotyping

We used the Phred–Phrap software (Ewing and Green, 1998; Ewing et al., 1998) to evaluate the quality of the electropherogram. We obtained consensus sequences from the alignment of the sense and antisense sequences of each strain using CAP3 software available at the web page Electropherogram quality analysis Phred (http://asparagin.cenargen.embrapa.br/phph/).

All HDV and HBV sequences were aligned and edited using the software BioEdit (v. 7.0.8) and the integrated CLUSTAL W program (Hall, 1999). HDV genotypes were classified by phylogenetic reconstructions using the published reference sequences from the GenBank database (http://www.ncbi.nlm.nih.gov/). Phylogenetic analyses were performed using a Bayesian approach, which was done using the Markov Chain Monte Carlo (MCMC) simulation implemented in BEAST v.1.6.1 (Drummond and Rambaut, 2007). The analysis was performed using relaxed uncorrelated lognormal molecular clock and GTR + G + I as nucleotide substitution model; MCMC chains were run for 10 million states, and sampled every 1000 runs to obtain the convergence of parameters. Maximum clade credibility tree was summarized after excluding 10% of burn-in using TreeAnnotator v.1.6.1 and the tree was visualized in FigTree v1.4.2. (Available at: http://tree.bio.ed.ac.uk/software/figtree).

3. Results

3.1. Demographical characteristics of HDV RNA positive individuals

Among the 92 individuals screened for anti-HD antibody, eight were positive (8.7%). Samples from these eight positive individuals were submitted to PCR. Half of them were positive for a fragment sequence of the delta antigen and submitted to sequencing. Only two of them (50%) had detectable HBV DNA and had the HBV subgenotype determined. Among the four HDV RNA carriers, just one was female. Their ages ranged from 23 to 49 years and they lived in three different municipalities (Table 1).

3.2. Genotype distribution

The four successful sequenced strains were submitted to phylogenetic analysis and showed that all sequences clustered, with high posterior probability, with the African genotype 8 together with other two strains found in Maranhão (Fig. 2). All sequences clustered in a unique branch of the tree apart from all the other described in GenBank. Although there were two infected individuals from the same municipality of Humberto de Campos, these sequences did no relate with each other in the tree. The same was found for the sample from Urbano Santos with the ones from our previous study (JF 298899_MA_Brazil e JF 298898_MA_Brazil) (Barros et al., 2011).

Among the four HDV-8 individuals co-infected with HBV, two had detectable HBV DNA by PCR and were sequenced. Both were classified into subgenotype D4 (Table 1).

The GenBank accession numbers for the four HDV sequences described in this study are KX599369–KX599372.

4. Discussion

In Brazil, HDV is a major public health burden in the Western Amazon region, where co-infection with HBV leads to severe forms of acute and chronic liver diseases (Bensabath et al., 1987; Braga et al., 2012; Viana et al., 2005). Conversely, only some cases of HDV infection have been sparsely found in other regions of Brazil (Mendes-Correa et al., 2011; Strauss et al., 1987). The Northeastern region of Maranhão, according to our knowledge, was the first region outside Amazon where a modest frequency of anti-HD serological marker (3.8%; 5/133) was found among positive HBsAg individuals. Also, the African HDV-8 was only found in this Brazilian region (Barros et al., 2011). Thus, this finding led us to investigate whether this genotype could be found in other municipalities of this state.

The HDV genotype 8 was firstly found infecting individuals in France who were born in West and Central Africa (Le Gal et al., 2006; Makuwa et al., 2008; Makuwa et al., 2009). In the present study, four strains were successfully sequenced and classified into

Download English Version:

https://daneshyari.com/en/article/3427834

Download Persian Version:

https://daneshyari.com/article/3427834

Daneshyari.com