Phylogenetic analysis of hepatitis delta virus isolated from HBsAg positive patients in Shahrekord, Iran

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OBJECTIVE: To find out the phylogenetic background of hepatitis delta virus (HDV) samples isolated in Shahrekord, Iran.

METHODS: A total of 350 hepatitis B surface antigen (HBsAg) positive sera samples were found from blood donors and HBsAg positive patients in blood transfusion center and clinical laboratory in Shahrekord, Iran. HDV RNA was extracted using RNXPlus (CinnaGen, Iran). A total of 421 bp corresponding to hepatitis delta antigen (HD Ag) gene have been isolated from HDV in Shahrekord, then were amplified in polymerase chain reaction system, sequenced for determining nucleotide sequence and compared with identified nucleotide sequences of these genes in other countries.

RESULTS: Among 350 HBsAg positive samples, we could detect HCV RNA in only two samples. After sequencing, the nucleotide sequences had a variability of 1/7–3/0 for HD Ag gene. The greatest sequence similarity existed between Iranian HD Ag sequence and JF694493-Iran, U25667-China with a sequence similarity of 99.7% and the least relationship between Iranian HD Ag sequence and AF008420-USA with a similarity of 92.9%.

CONCLUSIONS: It is suggested that precise genotype of HDV circulating in the region can be determined by more expansive sampling from different parts of Chahar mahal and Bakhtiari province and neigh bouring provinces (Esfehan and Khoozestan).

KEYWORDS
Hepatitis delta virus, Phylogenetic analysis, Sequencing, Shahrekord

1. Introduction

The hepatitis delta virus (HDV) (Delta) is a defective RNA pathogen which was first discovered in 1977. Around 1986, several other labs began to work on the molecular virology of this agent and over and over, HDV has provided us with intriguing and unique phenomena in molecular virology[1–3]. HDV requires helper functions from the hepatitis B virus (HBV) for virion assembly and propagation. Thus, HDV infection is necessarily associated with HBV infection because HDV ribonucleoprotein buds through the hepatitis B surface antigen (HBsAg) excretory pathway[4–9].

The HDV genome is a circular single–stranded RNA
HDV is now well known to induce a spectrum of both acute and chronic liver diseases\cite{9}. Superinfection with HDV in HBV carriers leads to more progressive chronic liver disease (80%), with higher incidence of cirrhosis and hepatocellular carcinoma\cite{10-13}.

More than 350 million individuals worldwide are HBV carriers and over 15 million people are infected with HDV worldwide and its prevalence in Italy, Eastern Europe and western region of Asia is higher than in the rest of the world and appears to be endemic in the Middle East\cite{8,14-16}.

The genetic diversity of HDV is related to the geographic origin of the isolates. Apart from HDV-1, which is ubiquitous, each virus clade is geographically localized: HDV-2 (previously labeled HDV-IIa) is found in Japan, Taiwan, and Yakoutia, Russia, HDV-4 (previously labeled HDV-IIIb) in Taiwan and Japan, HDV-3 in the Amazonian region, and HDV-5, HDV-6, and HDV-7 in Africa\cite{8,17}.

Genotype I has been associated with various types of liver disease, ranging from fulminant hepatitis to asymptomatic chronic HDV. Genotype II shows a less aggressive course, whereas genotype III causes a severe clinical course\cite{8,9}.

HDV genotyping has been performed by various methods such as hybridization, direct sequencing, and restriction fragment length polymorphism analysis of reverse transcription (RT) product of the HDV genome\cite{8,18}.

Phylogenetic analyses of HDV isolated in Iranian patients have been conducted previously. Because Iran is a large country with different ethnic groups, more data are required to have a better understanding of HDV characteristics. In the present study, delta antigen sequences of 5 HDV isolated from HBsAg positive patients, were analyzed to determine HDV genotype distribution\cite{9}.

2. Materials and methods

2.1. Serological tests

In this study, a total of 300 HBsAg positive sera samples that were found from blood donors and HBsAg positive patients in blood transfusion center and clinical laboratory in Shahrekord, Iran. All sera were checked for HBsAg using an ELISA kit (Enzygnost HBsAg 5.0 DADE BEHRINC). Anti-HDV antibody was explored using an ELISA kit (DIA-PRO, Italy) according to the manufacturer’s protocol.

2.2. RT–polymerase chain reaction (PCR) and PCR

HDV RNA was extracted from 200 µL serum using RNXPPlus (CinnaGen, Iran) as mentioned in the manufacturer’s protocol. Reverse transcriptase polymerase chain reaction (RT–PCR) was performed using a random hexamer 0.5 µL (0.2 µg/µL), a reverse primer as a GSP 1 µL (12.5 µmol/L), and an MMULV (Fermentas AB, Vilnius, Lithuania) in a final volume of 20 µL. About 2 µL of cDNA were amplified using 0.3 µmol/L forward and 0.3 µmol/L reverse primers:

F: 5’–TGC CAT GCC GAC CCG AAG AGG AA–3’
R: 5’– GGA GAG ACG GGA TCA CCG AAG AAG GAA GGC–3’ by

Taq DNA polymerase (Fermentase) at 94 °C for 5 min and 35 cycles: 94 °C for 40 seconds, 72 °C for 1 min (annealing and extension were at 72 °C), and ending at 72 °C for 5 min\cite{9}.

Finally, the PCR products were electrophoresed on a 2% agarose gel prepared in 1× Tris–borate–EDTA (TBE) buffer, stained with ethidium bromide, and evaluated under UV transilluminator. The PCR product with an expected length of 421 bp was analyzed in 1.5% agar gel electrophoresis.

2.3. Sequence analysis

The nucleotide sequences were edited using Edit View v.1.0.1 (Applied Biosics–ence, Australia). The 14 sequences regis-tered in GenBank (accession numbers JF694493–Iran, AF425644–Taiwan, U25667–China, AF309420–Japan, AJ309880–Russia, U81989–Ethiopia, U81988–Somalia, JX88135–Luxembourg, AB037949–Venezuela, KC590319–Brazil, L22065–South America, AF247965–Sweden, HF679406–France, M55042–Netherland) were aligned separately using the Clustal W v1.81 in order to obtain a consensus sequence. Subsequently, the sequences were analysed using the BioEdit package v.7.0.4.1 to compare the nucleotide sequences.

The Two nucleotide sequences of the Iranian IBR gB and gD genes were compared with the corresponding sequences from other regions of the world. Unrooted dendrogrammes were constructed using the Njplot software. Statistical support for dendrogrammes was obtained by boot–strapping using 1000 replicates.

3. Results

Among 350 HBsAg positive samples, we could only detect HCV RNA in two samples. The results are shown in Figure 1. The nucleotide sequences of the 421 bp fragments of the HD Ag gene from Iranian isolates were compared with the sequences of the HD Ag gene from the known reference sequences obtained from the GenBank nucleotide sequence

The nucleotide sequences had a variability of 1/7-3/0 for HD Ag gene (Table 1). The greatest sequence similarity existed between Iranian HD Ag sequence and JF694493-Iran, U25667-China with a sequence similarity of 99.7% and the least relationship between Iranian HD Ag sequence and AF008420-USA with a similarity of 92.9%. The results are shown in Table 1 and Figure 2.

Table 1 Sequence identity matrix of partial HD Ag gene of Iranian HDV isolates in comparison with other sequences.

| Sample       | Sample 1 ID | Sample 2 ID | JF694493-Iran ID | AF425644-Taiwan ID | U25667-China ID | AF309420-Japan ID | AJ309880-Russia ID | U81989-Ethiopia ID | U81988-Somalia ID | JX888135-Luxembourg ID | AB037949-Venezuela ID | KC590319-Brazil ID | M55042-Netherland ID | AF008420-USA ID |
|--------------|-------------|-------------|------------------|---------------------|-----------------|-------------------|---------------------|---------------------|-------------------|------------------------|----------------------|---------------------|---------------------|------------------|
| Sample 1     | 0.99        | 0.99        | 0.97             | 0.98                | 0.97            | 0.95              | 0.95                | 0.94                | 0.93              | 0.93                   | 0.92                 | 0.91                | 0.90                | 0.90             |
| Sample 2     | 0.99        | 0.97        | 0.99             | 0.99                | 0.98            | 0.96              | 0.94                | 0.93                | 0.92              | 0.92                   | 0.91                 | 0.91                | 0.91                | 0.90             |

**Figure 1.** Agarose gel electrophoresis of PCR products amplified. (Column M = 100 bp DNA Ladder, Columns 1 and 2 are positive samples.)

**Figure 2.** Dendrogramme based on sequence alignment analysis of two Iranian isolates and 15 of the reference isolates from other regions of the world for Delta gene of HDV.

4. Discussion

For the first time, HDV was known as a new nuclear antigen in hepatocytes patients infected with HBV. It was in conjunction with acute or chronic hepatitis. Then this antigen was called Delta antigen and was considered as gene products of HBV. Later, it was understood that Delta antigen is along with a transferable factor of disease and it is not from products of HBV genome. In patients liver or serum, HD Ag is found as two kinds based on number of amino acid or its weight. Small Delta antigen with weight of 24 000 KD and big Delta antigen with weight of 27 000 KD differ from each other in terms of 19 amino acids and
the sum of these two kinds is mRNA. HD Ag is important in forming structure of virus and enters nucleus of liver cells.

In this study, a piece with length of 412 open pairs from Delta antigen was used for philo genetic analysis of Delta virus. Infection with Delta virus is an infection which is seen throughout the world, but its prevalence is different in various geographical regions. Among 350 million people who are infected with HBV around the world, 5% suffers from both HBV and HDV. Geographical regions of the world has been divided to three groups (based on geographic spread): some regions with high epidemic such as Amazon in Venezuela, some African countries, regions such as south of Italy, Greece, Mediterranean countries, Bangladesh and some regions with low prevalence like developed countries which is seen among people with high risk like addicts injective drugs. RNA virus almost contains 1700 nucleotides. It is a single ring-shaped filament which is not structurally similar to HBV genome. Heterogeneity of virus is high and RNA virus in each person differs the other person in terms of sequence of nucleic acids. HD Ag is the only protein which is made by HDV and considered as internal part of virus structure.

Delta virus has one serotype and eight genotypes. Genotypes I, II, III are the most abundant ones based on geographical distribution. Therefore, genotype III is common in Southern America or among Yoopka natives in Venezuela country and causes acute diseases of liver. This genotype can cause Amazon black fever in lower region of Amazon river which is a kind of heavy hepatitis of this virus. Genotype II of Delta virus produces a milder disease in Eastern Asia (with a slower trend). Genotype I is common in Europe and Northern America so that produced disease is often fast or leads to progressive cirrhosis (with fast trend)[19].

In research done by Esmaeeli et al., on 26 patients infected with hepatitis delta in Tehran in 2009, all samples belonged to genotype I[9].

Mohabbi et al. conducted a study on 25 patients infected with hepatitis delta in 2008. And after philogenetic study, they specified that all samples belonged to genotype I of virus[21].

For determining frequency and genotype of HDV in patients infected with HIV and patients who were under dialysis, a study was done by Aghasadeghi et al. in 2013[22]. Among 70 people under study (120 patients under dialysis and 600 patients infected with HIV), all cases of positive HBsAg were reported. Nested PCR method was used for approving positive HDV–RNA in the samples and also anti–HDV. According to the result among 120 patients under dialysis about nine people (7.5%) and among 600 patients suffering from HIV about nine people (1.5%) showed positive HBsAg. According to nested PCR, three patients (33.3%) under dialysis and five patients (55.5%) suffering from HIV were positive in terms of anti–HDV. Totally, RNA of Delta virus was extracted from samples (37.5%), (2.5% patients under dialysis and 0.8% patients suffering from HIV). Positive samples were specified after philogenetic study and the sample belongs to clade I[22].

For studying outbreak of hepatitis delta and hepatitis B in blood donors without sign in Iran, research was conducted by Attaran et al. in 2013[23]. This research showed that among 854 people under study, positive HBsAg of 18 people (2%) were as positive anti–HDV and 154 people (18%) were HDV–DNA and 6% from whole samples was reported positive HDV–RNA. These results were approved by two methods: real–time PCR and seminested PCR. After philogenetic analysis of positive samples, it was specified that samples belong to HDV 1 group[23].

A research was done by Lee et al. in south of Taiwan in 2013[24]. Generally, 64 patients suffering from serum outbreak of anti–HDV Abs, HDV genotype, clinical demonstrations among patients with and without HDV infection. The result obtained from this study was that among 64 patients suffering from HIV, while about seven patients (10.9%) had HDV genome. After philogenetic analysis, it was specified that positive cases belonged to HDV 11[24].

In 2012, a research was done Mansour et al. in Mauritania for studying molecular epidemiology of hepatitis B and HBV in pregnant women and patients suffering from hepatitis[25]. In this study, 1200 pregnant
women and 946 hepatitis patients were considered. Among pregnant women and hepatitis patients, 10.6% and 18.3% had positive HBsAg, respectively. Also, anti–HBe Ab was reported (66.3%), and (76.5%) HDV–RNA was found in 10.1% of pregnant women and 17.3% of patients. After philogenetic analysis of positive samples, it was specified that common was 9.7%. This study approved high outbreak of HBV and HDV in Mauritania and genetic variety of virus in this region[25].

In addition, Mansour et al. conducted a research in 2012 for epidemiological study of hepatitis delta among blood donors of Nouakchott, Mauritania[26]. This study was done among 1 700 patients suffering from hepatitis with positive HBsAg. After studying and doing experiments, it was specified that 19.78% of donors were positive HDV Ab and 56 people (62.2%) were positive HDV–RNA. Philogenetic study showed that genotype 5 with 10.7% and genotype 1 with 89.3% had high outbreak in this region. It means that HDV in blood donors of Mauritania was one of the reasons of chronic hepatitis, liver cirrhosis or hepatocellular cancer of liver[26].

Le Gal et al. conducted a research in center and east of Turkey in 2012 for studying genetic variety on 34 patients of HBV/HDV[27]. Philogenetic study of piece 900–1 280 of genome HDV showed that all sides belonged to genotype I HDV. According to the results, two infections of HDV and HBV are very native in Turkey and both of them are repeated very much. Also, it has a wide genetic variety which may reflect evolution or outbreak of successive diseases[27].

In a study done by Barros et al. in 2011 on 133 Brazilian vectors of chronic HBsAg, after determining sequence, it was specified that genotype 2, 3 and 8 are the most common genotypes in this region[28].

In 2011, Kim et al. conducted a research among patients suffering from chronic hepatitis B to study synchronous infection outbreak in South Korea[29]. This research was conducted on 940 patients that three of them just had positive anti–HDV. After philogenetic study, all positive cases belonged to group 1 of HDV. This study showed that synchronous infection of HDV can not have significant clinical effect on Korean patients suffering from chronic infection of HBV[29].

In study which has been done by Foupouapouognigni et al. on 233 HBsAg Cameroonians in 2011, RNA of HBV was extracted from serum of HDV–Ab people[30]. In this study, among 233 patients with positive HBsAg, about 41 cases were diagnosed as positive HDV–Ab and RNA of Delta virus was indentified among 25 cases of them. Philogenetic analysis done on 25 samples showed that 22 samples (88%) of sterines belonged to genotype I[30].

In a study done by Gomes–Gouvêa et al. on 14 Brazilion suffering from sudden hepatitis in 2009, it was specified that all patients were positive in terms of having HBV DNA and HDV RNA[31]. Philogenetic analysis of HDV sequence showed that all positive cases belong to genotype III[31].

A study was done by Moriyama et al. on three Japanese patients suffering from chronic hepatitis B in 2005[32], RNA of HDV was separated from serum of these people. After comparing HDV genomic regions, it was specified that 80% of Miyako samples in Japan belong to genotype II b[32].

Saudy et al. conducted a research on 105 patients suffering from positive HBsAg in Greek in 2003[33]. According to this study, nine patients had positive HDV–Ab. And after extracting RNA, nucleotides within 853–1 265 were sequenced. Philogenetic analysis showed that 9 isolates of HDV belong to genotype I[33].

According to a study done by Ivaniushina et al. on 29 Russian patients with positive HBsAg in 2001, 14 patients were diagnosed positive in terms of HDV–Ag[34]. After philogenetic analysis, it was specified that 14 isolates of HDV belong to genotypes I and II[34].

In a study which was done by Sakugawa et al. on six Japanase patients suffering from chronic hepatitis in 1999, RNA HDV was separated from six people. Philogenetic study showed that genotype II b of this virus is common in Okinato, Japan[35].

In the present study, nucleotide sequence of coding–gene of Delta antigen from two positive serum samples was determined in Shahrekord and Philogenic proximity (similarity) of determined sequence was compared with positive sequence of this virus in gene bank. According to the results, samples under study had 99.1%–99.7% similarity to sequence of this virus from South Eastern Asian countries (China and Taiwan). We can put these viruses in phylum of genotype II of HDV. Perhaps, this genetic variety can be justified based on geographic spread of this virus. Because the amount of travels to American and European countries has been more limited in Iran, especially after victory of Islamic Revolution, so it seems that infection with this virus in the region under study (Shahrekord) has been brought from Southeast Asian countries (men go to these countries especially Japan and Korea for working).
Generally, it is suggested that precise genotype of HDV circulating in the region can be determined by more expansive sampling from different parts of Chahar mahal and Bakhtiari province and neighbour provinces (Esfehan and Khoozestan) and determining sequence of more positive samples and doing methods of molecular biology like RFLP and the other genotyping methods.

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Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Taylor JM. Structure and replication of hepatitis delta virus RNA. Curr Top Microbiol Immunol 2006; 307: 1–23.
[2] Taylor JM. Hepatitis delta virus. Virology 2006; 344(1): 71–76.
[3] Casey J, Cote PJ, Toshkov IA, Chu CK, Gerin JL, Hornbuckle WE, et al. Clevudine inhibits hepatitis delta virus viremia: a pilot study of chronically infected woodchucks. Antimicrob Agents Chemother 2005; 49(10): 4396–4399.
[4] Ghamari S, Alavian SM, Rizzetto M, Olivero A, Smedile A, Khedive A, et al. Prevalence of hepatitis delta virus (HDV) infection in chronic hepatitis B patients with unusual clinical pictures. Hepat Mon 2013; 13(8): e6731.
[5] Radjef N, Gordien E, Ivanushina V, Gautl E, Anaïs P, Dragan T, et al. Molecular phylogenetic analyses indicate a wide and ancient radiation of african hepatitis delta virus, suggesting a deltavirus genus of at least seven major clades. J Virol 2004; 78(5): 2537–2544.
[6] Kose S, Ece G, Gozaydin A, Turken M. Study on seroprevalence of hepatitis delta in a regional hospital in Western Turkey. J Infect Dev Ctries 2012; 6(11): 782–785.
[7] Kodani M, Martin A, Mixson-Hayden T, Drobeniuc J, Gish RR, Kamili S. One-step real–time PCR assay for detection and quantitation of hepatitis D virus RNA. J Virol Methods 2013; 193(2): 531–535.
[8] Alvarado–Mora MV, Locarnini S, Rizzetto M, Pinho JR. An update on HDV: virology, pathogenesis and treatment. Antivir Ther 2013; 18(3): 541–548.
[9] Esmaeili R, Alavian M, Hajibeigi B, Sabouri E, Edalat R, Adeli A, et al. Phylogenetic analysis of twenty-six cases of hepatitis delta virus isolates in Tehran, Iran. Hepat Mon 2009; 9(3): 196–200.
[10] Sheldon J, Ramos B, Toro C, Rios P, Martínez–Alarcón J, Bottecchia M, et al. Does treatment of hepatitis B virus (HBV) infection reduce hepatitis delta virus (HDV) replication in HIV–HBV–HDV–coinfected patients? Antiviral Ther 2008; 13(1): 97–102.
[11] Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. Gut 2009; 46: 420–426.
[12] Alavian MS, Alavian HS. Hepatitis D virus infection; Iran, Middle East and Central Asia. Hepat Mon 2005; 5(4): 137–143.
[13] Ghamari S, Alavian SM, Rizzetto M, Olivero A, Smedile A,
Khedive A, et al. Prevalence of hepatitis delta virus (HDV) infection in chronic hepatitis B patients with unusual clinical pictures. Hepat Mon 2013; 13(8): e6731.

[14] Zaidi G, Idrees M, Malik FA, Amin I, Shahid M, Younas S, et al. Prevalence of hepatitis delta virus infection among hepatitis B virus surface antigen positive patients circulating in the largest province of Pakistan. J Med Virol 2010; 7: 283.

[15] Rizzetto M, Ciancio A. Epidemiology of hepatitis D. Semin Liver Dis 2012; 32(3): 211–219.

[16] Popescu GA, Otelea D, Gavriliu LC, Neaga E, Popescu C, Mohebbi SR, Zali N, Derakhshan F, Tahami A, Mashayekhi R, Amini-Bavil-Olyaee S, et al. Molecular epidemiology of hepatitis delta virus genotypes in outbreaks of fulminant hepatitis (Labrea black fever) in the western Brazilian Amazon region. J Gen Virol 2009; 80: 2092–2099.

[17] Aghasasedehi MR, Mohraz M, Bahramali G, Aghakhani A, Banifazl M, Foroughi M, et al. Frequency and genotype of hepatitis D virus infection in patients infected with hepatitis B virus in Bucharest: a cross-sectional study. J Med Virol 2013; 85(5): 769–774.

[18] Le Gal F, Gault E, Ripault M, Serpaggi J, Trinchet J, Gordion E, et al. Eighth major clade for hepatitis delta virus. Emerg Infect Dis 2006; 12(9): 1447–1450.

[19] Mohlebhi SR, Zali N, Derakhshan F, Tahami A, Mashayekhi R, Amini–Bavil–Olyaei S, et al. Molecular epidemiology of hepatitis delta virus (HDV) in Iran: a preliminary report. J Med Virol 2008; 80: 2092–2099.

[20] Aghasasedehi MR, Mohraz M, Bahramali G, Aghakhani A, Banifazl M, Foroughi M, et al. Frequency and genotype of hepatitis D virus infection in patients infected with HIV and those undergoing hemodialysis. Hepat Mon 2013; 13(5): e7481.

[21] Attaran MS, Sharifi Z, Hosseini SM, Samei S, Ataei Z. Prevalence of hepatitis B and hepatitis D coinfection in asymptomatic blood donors in Iran. APMIS 2014; 122(3): 243–247.

[22] Lee CY, Tsai HC, Lee SS, Wu KS, Sy CL, Chen JK, et al. Higher rate of hepatitis events in patients with human immunodeficiency virus, hepatitis B, and hepatitis D genotype II infection: a cohort study in a medical center in Southern Taiwan. J Microbiol Immunol Infect 2013; pii: S1684-1182(13)00146-1. doi: 10.1016/j.jmii.2013.08.001.

[23] Mansour W, Malick FZ, Sidiya A, Ishagh E, Chekaraou MA, Veillon P, et al. Prevalence, risk factors, and molecular epidemiology of hepatitis B and hepatitis delta virus in pregnant women and in patients in Mauritania. J Med Virol 2012; 84(8): 1186–1198.

[24] Mansour W, Bollahi MA, Hamed CT, Brichler S, Le Gal F, Ducancelle A, et al. Virological and epidemiological features of hepatitis delta infection among blood donors in Nouakchott, Mauritania. J Clin Virol 2012; 55(1): 12–16.

[25] Le Gal F, Badur S, Hawajri NA, Akyüz F, Kaymakoglu S, Brichler S, et al. Current hepatitis delta virus type 1 (HDV1) infections in central and eastern Turkey indicate a wide genetic diversity that is probably linked to different HDV1 origins. Arch Virol 2012; 157(4): 647–659.

[26] Barros LM, Gomes–Gouveia MS, Pinho JR, Alvarado–Mora MV, Dos Santos A, Mendes–Corrêa MC, et al. Hepatitis delta virus genotype 8 infection in Northeast Brazil: inheritance from African slaves? Virus Res 2011; 160(1–2): 333–339.

[27] Kim HS, Kim SJ, Park HW, Shin WG, Kim KH, Lee JH, et al. Prevalence and clinical significance of hepatitis D virus co-infection in patients with chronic hepatitis B in Korea. J Med Virol 2011; 83(7): 1172–1177.

[28] Foupouapogognigni Y, Noah ND, Sartre MT, Njouom R. High prevalence and predominance of hepatitis delta virus genotype 1 infection in Cameroon. J Clin Microbiol 2011; 49(3): 1162–1164.

[29] Gomes–Gouveia MS, Soares MC, Bensabath G, de Carvalho–Mello IM, Brito EM, Souza OS, et al. Hepatitis B virus and hepatitis delta virus genotypes in outbreaks of fulminant hepatitis (Labrea black fever) in the western Brazilian Amazon region. J Gen Virol 2009; 90: 2638–2643.

[30] Moriyama M, Taira M, Matsumura H, Aoki H, Arakawa Y, Kaneko M, et al. Full genomic analysis of hepatitis delta virus prevalent on Miyako Island, Japan. Intervirology 2005; 48(6): 246–254.

[31] Saudy N, Sugauchi F, Tanaka Y, Suzuki S, Aal AA, Zaid M, et al. Genotypes and Phylogenetic characterization of hepatitis B and delta viruses in Egypt. J Med Virol 2003; 70: 529–536.

[32] Ivaniushina V, Radjef N, Alexeeva M, Gault E, Semenov êa MS, Pinho JR, Alvarado–Mora MV, Dos Santos A, Mendes–Corrêa MC, et al. Hepatitis delta virus genotype 8 infection in Northeast Brazil: inheritance from African slaves? Virus Res 2011; 160(1–2): 333–339.

[33] Kim HS, Kim SJ, Park HW, Shin WG, Kim KH, Lee JH, et al. Prevalence and clinical significance of hepatitis D virus co-infection in patients with chronic hepatitis B in Korea. J Med Virol 2011; 83(7): 1172–1177.

[34] Barros LM, Gomes–Gouveia MS, Pinho JR, Alvarado–Mora MV, Dos Santos A, Mendes–Corrêa MC, et al. Hepatitis delta virus genotype 8 infection in Northeast Brazil: inheritance from African slaves? Virus Res 2011; 160(1–2): 333–339.

[35] Moriyama M, Taira M, Matsumura H, Aoki H, Arakawa Y, Kaneko M, et al. Full genomic analysis of hepatitis delta virus prevalent on Miyako Island, Japan. Intervirology 2005; 48(6): 246–254.

[36] Saudy N, Sugauchi F, Tanaka Y, Suzuki S, Aal AA, Zaid M, et al. Genotypes and Phylogenetic characterization of hepatitis B and delta viruses in Egypt. J Med Virol 2003; 70: 529–536.