J Microbiol Immunol Infect 2010;43(6):519–523

Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

Journal homepage: http://www.e-jmii.com

Short Communication

Seroprevalence of Hepatitis D Virus and its Risk Factors in the West of Iran

Amir Houshang Mohammad Alizadeh[a]*, Mitra Ranjbar[b], Ali Shabahang Saber Tehrani[a], Fariba Keramat[c], Moggan Mamani[c], Mehdi Rezazadeh[c], Peyman Aini[c], Alireza Khalilian[c], Amir Majlesi[c], Mehrdad Hajilooi[c]

[a]Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

[b]Iran University of Medical Sciences, Iran.

[c]Hamedan University of Medical Sciences, Iran.

Despite the decreasing trend in hepatitis D virus (HDV) infection worldwide, the importance of this disease cannot be underestimated. The aim of this study was to evaluate patients positive for HBsAg with respect to HDV infection and related factors. Patients with chronic hepatitis B who presented at Hamedan Province Hepatitis Community Center in 2002–2007 were included. A questionnaire covering demographic variables and history of hepatic disease was completed for each patient. Necessary tests were performed and antibodies to HDV were measured using an enzyme-linked immunosorbent assay. Of 81 HBsAg positive patients, 14 (17.3%) contained anti-HDV IgG. Only one of the patients with anti-HDV IgM was positive for HBsAg. Of the anti-HDV IgG positive patients, two (14.3%) were women. Among the women examined in this study, 24 (35.8%) were anti-HDV IgG negative ($p=0.21$), and of these, six (42.8%) were HBeAg positive while 17 (25.4%) of the anti-HDV IgG negative women were positive for HBeAg ($p=0.16$). The prevalence of chronic hepatitis B among anti-HDV IgG positive and negative patients was 28.6% and 39.2% respectively ($p=0.31$). Because of the relatively high rate of hepatitis B virus (HBV) and HDV co-infection in our study subjects, it is vital that healthcare providers and policy makers to recognize the risk factors associated with this HBV and HDV co-infection as well as the reasons for this increased anti-HDV serology in HBV carriers.

KEYWORDS: HBeAg, hepatitis B virus, hepatitis D virus, Iran

*Corresponding author. Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, 7th floor, Taleghani Hospital, Parvaneh Ave, Tabnak Str, Evin, Tehran 19857, Iran. P.O. Box: 19835-178

E-mail: ahmaliver@yahoo.com

Article History:
Received: Aug 18, 2008
Revised: Dec 15, 2008
Accepted: Jan 7, 2009

Introduction

Hepatitis D virus (HDV) is a transmissible agent discovered in 1977.[1] It requires helper functions from hepatitis B virus (HBV) for virion assembly and propagation.[2] This unique human virus is associated with co-infections or super infections of patients already infected with HBV. Coexisting infection with HDV tends to accelerate the progress of chronic HBV infection towards chronic hepatitis, cirrhosis and hepatocellular carcinoma.[3] Hepatitis

caused by HDV may result in a 3-fold increase in hepatocellular carcinoma development and 2-fold increase in mortality compared with HBV infection without HDV.[4]

HBV infection is a global health problem; over 350 million people worldwide are chronic carriers of the virus. It has been estimated that approximately 5% of the global HBsAg carriers are also co-infected with HDV. Thus there are 10–15 million HDV carriers worldwide.[5,6] The geographic distribution of HDV infection might be expected to mirror that of HBV. Nevertheless, the rate of HDV infection is not a simple reflection of HBV infection, as areas endemic for HBV may be almost HDV-free.[7] HDV prevalence in Italy, Eastern Europe and western Asia is higher than the rest of the world[8] with infection apparently endemic in the Middle East.[9]

High prevalence areas of HDV infection include Italy, parts of Eastern Europe, the Amazon basin, Venezuela, Columbia, some Pacific Islands, Pakistan and Western Asia.[7,10–13] It is estimated that each year, 7,500 HDV infections occur in the United States.[11] New foci of infection have been identified on the island of Okinawa in Japan,[14] villages in China, northern India[15] and southern Albania.[16] The subtropical area in South America remains an important potential reservoir for new outbreaks of HDV infection.[17] From different studies in Iran, 2.4–5.8% of HBV carriers were reportedly HDV positive.[18–20] Changes in HDV epidemiology have occurred over the past 10 years with several studies suggesting that the prevalence of HDV infection has declined over the past decades.[8,21]

Overall, HDV infection is still an important public health problem worldwide and remains a major cause of mortality and liver transplantation. The purpose of this study was to evaluate the risk factors and prevalence of HDV in HBV-infected individuals in Iran.

Methods

A cross-sectional study was conducted in the Hamedan province of Iran from 2002 to 2007. All HBV-infected individuals enlisted in the Hamedan hepatitis association from January 2002 to January 2007 who were willing to sign informed written consent participated in the study. A questionnaire including age, sex, history of intravenous drug abuse, history of blood transfusion, mother carrier status of hepatitis B, liver function tests, chronicity or carrier state was administered. Abnormality in alanine transaminase levels for at least 6 months in HBsAg positive patients was considered to be chronic hepatitis B.

Approximately 5 mL of blood was collected from each participant. Serum samples were tested for the presence of anti-HDV antibodies (IgM, IgG) using an HDV antibody enzyme-linked immunosorbent assay (ETI-AB-DELTAK-2; Diasorin, Dietzenbach, Germany). Other laboratory tests such as complete blood count and liver function tests (aspartate transaminase, alanine transaminase, alkaline phosphatase, bilirubin and prothrombin time) were also performed. The study was approved by the ethics committee of the Hamedan University of Medical Sciences in Hamedan, Iran.

Statistical analysis

Data analysis was performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Qualitative or quantitative variables were analyzed using non-parametric tests, the χ^2 test and Fisher's exact test. Quantitative variables were also analyzed with the Student's t test. A p value < 0.05 was considered significant.

Results

Of the 81 HBsAg-positive individuals tested for the presence of anti-HDV antibodies, 55 (67.9%) were males. The average age was 35.6 ± 14.7 years and most (61.7%) were under 30 years old. Thirty participants (37.0%) suffered from chronic hepatitis and the rest were carriers. Anti-HDV IgG was found in 14 (17.3%) individuals. Only one participant was positive for anti-HDV IgM. Female constituted 14.3% of the 14 anti-HDV IgG positive participants and 35.8% of the anti-HDV negative participants. The difference was not statistically significant ($p = 0.21$).

Factors such as age, sex, the mother's carrier state of hepatitis B, history of blood transfusion, history of intravenous drug abuse, chronic hepatitis or carrier state, liver enzymes and positive HBeAg serology were analyzed to determine a correlation with positive or negative anti-HDV serology. Of the above mentioned factors, only intravenous drug abusers showed a statistically significant difference between the two groups. The Table shows the relationship of different factors to anti-HDV serology.

Table. Age, sex, chronic hepatitis or carrier state, liver enzymes and positive HBeAg serology compared with anti-hepatitis D virus positive and negative groups[a]

Characteristics	anti-HDV IgG positive (n=14)	anti-HDV IgG negative (n=67)	p
Sex, male	12 (85.7)	43 (64.2)	0.41
Age (yr)	36.9±12.6	35.4±15.6	0.77
Mother's carrier state	9 (64.2)	24 (35.8)	0.03
Blood transfusion	1 (7.1)	10 (14.9)	0.21
Intravenous drug abuser	2 (14.3)	5 (7.4)	0.21
HBeAg positive	6 (42.8)	17 (25.4)	0.16
Chronic hepatitis B	4 (28.6)	26 (38.8)	0.31
Hepatitis B carrier	10 (71.4)	38 (58.5)	0.41
Serum ALT (IU/L)	40.1±26.0	55.6±38.0	0.37
Serum AST (IU/L)	32.9±9.9	41.9±22.0	0.34

[a]Data presented as n (%) or mean±standard deviation. IgG=immunoglobulin G; ALT=alanine transaminase; AST=aspartate transaminase.

Discussion

This study demonstrates that the prevalence of anti-HDV seropositivity (17.3%) in the Hamedan province of Iran is higher than the level reported by the World Health Organization (5%). It is also higher than previous studies carried out in this region[19] and those carried out in other parts of Iran.[18,21] This may be due to the high number of intravenous drug abuse subjects in our study.

In a study carried out in Tehran in 1988, 2.5% of HBV carriers (n=120) were reported to be HDV positive.[18] Amini et al found this proportion to be 2.4% in Hamedan in 1993 (n=123).[11] In a more recent study in 2005, 5.8% of HBsAg positive individuals were found to be HDV infected in Golestan province (n=164).[20]

Other studies conducted in the Middle East region have reported different results. A study by Njoh et al[22] in Saudi Arabia found that 13.6% of HBV carriers (n=81) were also positive for anti-HDV. In Pakistan (n=246)[23] and Turkey (n=889)[24], 26.8% and 6%, respectively, were HBV carriers and HDV positive.

There are areas with high prevalence of HBV infection but relatively low prevalence, if any, of HDV infection, suggesting that other factors, such as age at HBV infection, may determine the acquisition of HDV infection. As an example, for Alaskan Natives in which HBV infection occurs during infancy and childhood, the prevalence of HDV infection is negligible despite the endemic nature of HBV. HDV appears to be endemic in the Middle East, but again its distribution bears little relationship to that of HBV.[25] In this study, the prevalence of anti-HDV seropositivity was higher in males compared with females. Risk factors such as a greater possibility of multiple partners among males compared with females in Islamic countries like Iran, and intravenous drug use can be explained as possible causes of this discrepancy.

Some countries have witnessed a declining trend in the prevalence of HDV infection.[10,18,26,27] HDV was found to be responsible for a high proportion of cases of HBV-related acute and chronic liver disorders in southern Europe during the 1970s. However, by the 1990s, its seroprevalence had substantially declined.[28] In Italy, the prevalence of anti-HDV among HBsAg carriers with liver diseases decreased from 25% in 1983 to 14% in 1992.[28] A multicenter Italian study[29] conducted in 1997 reported HDV positivity of only 8.3% in HBsAg positive patients — a figure much lower than those observed in the previous two multicenter surveys performed in 1987 and 1992 (23% and 14%, respectively). The authors estimated that from 1987 to 1997, the rate of decrease in the proportion of HBsAg carriers with anti-HDV was approximately 1.5% per year.[29] A similar decrease (from 15.1% in 1983 to 7.1% in 1992) has also been reported in Spain. In Taiwan, Huo et al[10] reported a decrease in HDV infection from 23.7% in 1983 to 4.2% in 1995.

Improvements in socioeconomic conditions, an increased awareness of the risk of transmitting infectious

diseases fostered by acquired immunodeficiency syndrome prevention policy, promotion of disposable needles and aggressive vaccination campaigns against HBV have assisted in the control of HBV infection and the spread of HDV infection.[30]

Most notably, this study shows a dramatic increase in positive HDV serology (17.3%) among HBV carriers compared with the study conducted in the same region almost a decade ago. Amini et al found this to be 2.4% in Hamedan in 1993 ($n=123$).[19] Considering the declining trend of HDV in the world, the reasons behind this increase are yet to be determined and further studies in this field are required.

In this study, factors such as age, sex, chronic hepatitis or carrier state, liver enzymes and positive HBeAg serology did not have any association with positive anti-HDV serology, which corresponds to the previous studies carried out in this field.[25,31] There was a significant association between HDV positivity and the mother's hepatitis B carrier status. One of the most significant routes of transmission for hepatitis B in Iran may be the horizontal route such as household contact. Following mandatory active immunization of newborns since 1993, vertical transmission of hepatitis B has dramatically dropped. It is thought that most patients with a history of mother carrier state of hepatitis B were born before the commencement of national immunization against hepatitis B, or they received the HBsAg via the horizontal route. This study needs to be repeated with a larger population to confirm the relationship between HDV antibody positivity and HBsAg positivity in mothers.

In conclusion, it is of utmost importance for healthcare providers and policy makers to recognize the risk factors associated with HBV and HDV co-infection and the reasons behind this regional increase in anti-HDV serology in HBV carriers, to control and contain this health problem.

References

1. Rizzetto M, Canese MG, Arico S, Crinelo O, Trepo C, Bonino F, et al. Immunofluorescence detection of a new antigen-antibody system (delta/anti-delta) associated to hepatitis B virus in liver and serum of HBsAg carriers. *Gut* 1997;18:997–1003.
2. Sureau C, Guerra B, Lanford RE. Role of the large hepatitis B virus envelope protein in infectivity of the hepatitis delta virion. *J Virol* 1993;67:366–72.
3. Toukan AU, al-jKandari S. The role of hepatitis D virus in liver disease in the Middle East. *Prog Clin Biol Res* 1991;364: 63–8.
4. Fattovich G, Giustina G, Christensen E, Pantelena M, Zagni I, Realdi G, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. *Gut* 2000;46:420–6.
5. Hadziyannis SJ. Decreasing prevalence of hepatitis D virus infection. *J Gastroenterol Hepatol* 1997;12:745–6.
6. Farci P. Delta hepatitis: an update. *J Hepatol* 2003;39(Suppl 1): S212–9.
7. Rizzetto M, Hadziyannis S, Hansson BG, Toukan A, Gust I. Hepatitis delta virus infection in the world: epidemiological patterns and clinical expression. *Gastroenterol Int* 1992;5:18–32.
8. Tapalaga D, Forzani B, Hele C, Paravacini O, Ponzetto A, Theilmann L. Prevalence of the hepatitis D virus in Rumania. *Hepatogastroenterology* 1986;33:238–9.
9. Gaeta GB, Stroffolini T, Chiaramonte M, Ascione T, Stornaiuolo G, Lobello S, et al. Chronic hepatitis D: a vanishing disease? An Italian multicenter study. *Hepatology* 2000;32:824–7.
10. Casey JL, Brown TL, Colan EJ, Wignall FS, Gerin JL. A genotype of hepatitis D virus that occurs in northern South America. *Proc Natl Acad Sci USA* 1993;90:9016–20.
11. Anonymous. Centers for Disease Control. Hepatitis surveillance report. MMWR 1990;53:23.
12. Rizzetto M, Ponzetto A, Forzani I. Epidemiology of hepatitis delta virus: overview. *Prog Clin Biol Res* 1991;364:1–20.
13. Mumtaz K, Hamid SS, Adil S, Afaq A, Islam M, Abid S, et al. Epidemiology and clinical pattern of hepatitis delta virus infection in Pakistan. *J Gastroenterol Hepatol* 2005;20:1503–7.
14. Sakugawa H, Nakasone H, Shokita H, Nakayoshi T, Kinjo F, Saito A, et al. Seroepidemiological study of hepatitis delta virus infection in Okinawa, Japan. *J Med Virol* 1995;45:312–5.
15. Singh V, Goenka MK, Bhasin DK, Kochhar R, Singh K. A study of hepatitis delta virus infection in patients with acute and chronic liver disease from northern India. *J Viral Hepat* 1995; 2:151–4.
16. Dalekos GN, Zervou E, Karabini F, Tsianos EV. Prevalence of viral markers among refugees from southern Albania: increased incidence of infection with hepatitis A, B and D viruses. *Eur J Gastroenterol Hepatol* 1995;7:553–8.
17. Manock SR, Kelley PM, Hyams KC, Douce R, Smalligan RD, Watts DM, et al. An outbreak of fulminant hepatitis delta in the Waorani, an indigenous people of the Amazon basin of Ecuador. *Am J Trop Med Hyg* 2000;63:209–13.
18. Rezvan H, Forouzandeh B, Taroyan S, Fadaiee S, Azordegan F. A study on delta virus infection and its clinical impact in Iran. *Infection* 1990;18:26–8.
19. Amini S, Mahmoodi MF, Andalibi S, Solati AA. Seroepidemiology of hepatitis B, delta and human immunodeficiency virus infections in Hamadan province, Iran: a population based study. *J Trop Med Hyg* 1993;96:277–87.

20. Gholamreza R, Shahryar S, Abbasali K, Hamidreza J, Abdolvahab M, Khodaberdi K, et al. Seroprevalence of hepatitis B virus and its co-infection with hepatitis D virus and hepatitis C virus in Iranian adult population. *Indian J Med Sci* 2007;61:263–8.
21. Huo TI, Wu JC, Lin RY, Sheng WY, Chang FY, Lee SD. Decreasing hepatitis D virus infection in Taiwan: an analysis of contributory factors. *J Gastroenterol Hepatol* 1997;12:747–51.
22. Njoh J, Zimmo S. Prevalence of antibody to hepatitis D virus among HBsAg-positive drug-dependent patients in Jeddah, Saudi Arabia. *East Afr Med J* 1998;75:327–8.
23. Zuberi BF, Quraishy MS, Afsar S, Kazi LA, Memon AR, Qadeer R, et al. Frequency and comparative analysis of hapatitis D in petients seeking treatment for hepatitis B. *J Coll Physicians Surg Pak* 2006;16:581–4.
24. Celen MK, Ayaz C, Hosoglu S, Geyik MF, Ulug M. Anti-hepatitis delta virus seroprevalence and risk factors in patients with hepatitis B in Southeast Turkey. *Saudi Med J* 2006;27:617–20.
25. Toukan AU, al-Kandari S. The role of hepatitis D virus in liver disease in the Middle East. *Prog Clin Biol Res* 1991;364:63–8.
26. Sagnelli E, Stroffolini T, Ascione A, Chiaramonte M, Craxi A, Giusti G, et al. Decrease in HDV endemicity in Italy. *J Hepatol* 1997;26:20–4.
27. Navascues CA, Rodriguez M, Sotorrio NG, Sala P, Linares A, Suarez A, et al. Epidemiology of hepatitis D virus infection: changes in the last 14 years. *Am J Gastroenterol* 1995;90:1981–4.
28. Smedile A, Lavarini C, Farci P, Arico S, Marinucci G, Dentico P, et al. Epidemiologic patterns of infection with the hepatitis B virus-associated delta agent in Italy. *Am J Epidemiol* 1983;117: 223–9.
29. Jacobson IM, Dienstag JL, Werner BG, Brettler DB, Levine PH, Mushahwar IK. Epidemiology and clinical impact of hepatitis D virus (delta) infection. *Hepatology* 1985;5:188–91.
30. Stroffolini T, Ferrigno L, Cialdea L, et al. Incidence and risk factors of acute delta hepatitis in Italy: Results from a national surveillance system. *J Hepatol* 1994;21:113.
31. Hsieh T, Liu C, Chen D, Chen P. Natural Course and Treatment of Hepatitis D Virus Infection. *J Formos Med Assoc* 2006;105: 869–81.