

The Prevalence and Risk Factors of Hepatitis Delta Virus in HIV/HBV Co-Infected Patients in Shiraz, Iran, 2012

Mohammad Motamedifar^{1,2}, PhD;
Mohammad Taheri², MSc;
Kamran Bagheri Lankarani³, MD;
Mina Gholami², BC;
Mahmood Amini Lari¹, MD;
Hossein Faramarzi¹, MD;
Jamal Sarvari², PhD

¹Shiraz HIV/AIDS Research Center (SHARC), Shiraz University of Medical Sciences, Shiraz, Iran;

²Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran;

³Health Policy Research Center (HPRC), School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:

Jamal Sarvari, PhD;
Assistant Professor of Virology,
Department of Bacteriology and
Virology, School of Medicine,
Shiraz University of Medical Sciences,
P.O. Box: 71348- 45794, Shiraz, Iran

Tel/Fax: +98 71 32304356
Email: sarvarij@sums.ac.ir

Received: 06 May 2014

Revised: 23 June 2014

Accepted: 13 July 2014

Abstract

Evidence has shown that liver disease caused by hepatitis viruses can be more aggressive and severe in HIV infected subjects. Therefore, the present cross-sectional study aimed to evaluate the seroprevalence of HDV infection among HIV/HBV co-infected clients in Shiraz, southwest Iran. In this study, 178 patients co-infected with HBV and HIV individuals were enrolled. The diagnosis of HIV infection was documented based on serological assays. The demographic and complementary data were collected by a questionnaire. HBsAg and HDV Ab were detected by commercial quantitative enzyme linked immunosorbent assay kits according to the manufacturer's instructions. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured. The mean age of the participants was 37.4 ± 7.4 years (range 22-63). 175 (98.4 %) patients were male and 3 (1.6 %) were female. Among 178 patients co-infected with HIV/HBV, 35 cases (19.7%, 95% CI: 14%-25%) were anti-HDV positive and 143 (80.3%) were negative for anti-HDV. HDV exposure in HIV/HBV co-infected patients was associated with blood transfusion ($P=0.002$, OR: 14.3) and prison history ($P=0.01$, OR: 2.31) but not with age, marital status, unsafe sex contact, and injection drug abuse. Our data showed a relatively high prevalence of HDV infection in HIV infected population in Shiraz, Iran. The high frequency of HDV Ab in patients with blood transfusion and prison history reveals that HDV transmission occurs more frequently in the parental route than sexual contacts; therefore, blood screening for HDV diagnosis in the high-risk group is recommended.

Please cite this article as: Motamedifar M, Taheri M, Lankarani KB, Gholami M, Lari MA, Faramarzi H, Sarvari J. The Prevalence and Risk Factors of Hepatitis Delta Virus in HIV/HBV Co-Infected Patients in Shiraz, Iran, 2012. Iran J Med Sci. 2015;40(5):448-453.

Keywords • Prevalence • Hepatitis delta virus • HIV • Hepatitis B virus

Introduction

Hepatitis delta virus (HDV) is a defective sub-viral agent, which requires the hepatitis B virus surface antigen (HBsAg) to be complete and infective.¹ It was discovered by Rizzetto in 1977 among patients with a severe form of hepatitis B virus (HBV) infection. It has a small (1.7 Kb) single stranded, circular RNA genome surrounded by a small 24-kDaHDAG and a large 27-kDa HDAG antigens. Viral particle comprises of an RNA molecule enclosed by delta antigens, which is surrounded by a HBsAg

containing envelope.¹ Worldwide, approximately, 15-20 million individuals infected with HDV represents nearly 5% of all individuals with chronic hepatitis B.^{2,3} Mediterranean countries, Eastern Europe, Middle East, Central Asia, Central Africa, and the Amazonian basin are considered as endemic areas.⁴ The highest prevalence of HDV has been detected in some parts of Africa, South America, Romania, Russia, and the Mediterranean region, including Southern Italy while it is uncommon in Northern Europe and North America.^{4,5} In Iran, although the overall prevalence of HDV among HBsAg positive carriers is around 5%,⁵ reports from different areas showed various prevalence from 2-17.3%.⁶⁻⁹ Few surveys from Iran have reported the prevalence of HDV in high-risk groups including hemophilia and human immunodeficiency virus (HIV) infected individuals.^{8,10} Frequent consequence of chronic HBV-HDV coinfection is a severe and quick progressive liver disease that is hardly treatable.¹¹ Two epidemiological patterns with different outcomes including co-infection (20% of cases) and super-infection (80% of cases) have been described for HDV infection.¹² Co-infection usually results in an acute self-limited illness with increasing risk of fulminant hepatitis, whereas super-infection generally causes severe and rapidly progressive liver disease that often leads to end-stage liver disease including cirrhosis and hepatocellular carcinoma.¹³ In general, only 15% of the infected patients with HBV in comparison with 70-80% of HDV-infected patients end up with cirrhosis.¹⁴ Evidence showed that liver disease caused by hepatitis viruses can be more aggressive and severe in HIV carriers, leading to cirrhosis and liver failure in a shorter period of time.^{1,15} Also, HIV-infected patients are more likely to be exposed to factors precipitating decompensated liver disease, such as infections (cytomegalovirus, mycobacteria, etc.), alcohol, and hepatotoxic drugs, such as ketoconazole, cothrimoxazole, antituberculous drugs, phenytoin, benzodiazepines, and antiretroviral agents.^{1,15} Moreover, HIV-infected individuals are at a higher risk for acquiring HDV as the same route of transmission of these viruses (sexually and parenteral),¹ therefore, surveying the prevalence of hepatitis viruses in HIV infected individuals is more important. The prevalence of HDV-HBV co-infection, in HIV patients, was reported to be in a range from 1.2% in southeast Brazil to 14.5% in south and east of Europe, and 22.2% in Taiwan.⁸ In Iran, the prevalence of HDV in individuals with HIV/HBV co-infection has been reported between 0.012-31.57%.^{16,17} However, limited information is available

regarding the seroprevalence and epidemiology of HDV among HIV/HBV co-infected patients in Iran. Thus, the present cross-sectional study aims to evaluate the seroprevalence of HDV infection among HIV/HBV co-infected clients referring to the Shiraz HIV referral center.

Participants and Methods

All available patients were recruited based on convenience sampling from 2004 to 2012 from the HIV research center, Shiraz, Iran. The diagnosis of HIV infection was documented based on serological assays, including the detection of HIV antibody using enzyme immunoassay that was confirmed using immunoblot assay. The demographic data including age, sex, HIV transmission risk factors, CD4 count, and other complementary information were collected by a questionnaire. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences and written informed consent was obtained from each participant before sampling.

Sampling

A blood sample of 5 mL was taken from each participant and allowed to clot for 3 hours. Then, the samples were spun at 3000 rpm for 10 minutes and the serum was separated, aliquoted, and stored at -20°C until assayed.

ELISA Tests

HBsAg (Dia.Pro, Italy) and HDV Ab (Dia.Pro, Italy) were detected by commercial quantities enzyme linked immunosorbent assay kits according to the manufacturer's instructions.

ALT, AST and CD4 Assay

Alanine aminotransferase and aspartate aminotransferase were measured using a standardized kit (Bionik, Tehran, Iran). All patients with abnormal alanine aminotransferase and aspartate aminotransferase results were investigated in this study. CD4 cell counts were measured using flow cytometry instrument (CyFlow®, GMBH, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS (version 16.0; SPSS Inc., Chicago, IL, USA). In addition to the descriptive test, crosstab test and Chi-square test were used to compare different variables and to find out the association of two-way tables. P values ≤ 0.05 were considered significant. Multivariate analysis was carried out using the logistic regression method.

Results

In this study, 178 patients co-infected with HBV and HIV, among 1,480 HIV infected individuals were recruited. The mean age of the participants was 37.4 ± 7.4 years (range 22-63). 175 (98.4%) patients were male and 3 (1.6%) were female. 28 (15.7%) were married and 150 (84.3%) were single. Among 178 patients co-infected with HIV/HBV, 35 (19.7%, 95% CI: 14%-25%) cases were positive for HDV Ab and 143 cases (80.3%) were negative for anti-HDV.

Table 1 summarizes the main characteristics of the study population by HDV status. The results of multivariate analysis demonstrated that HDV exposure was independently associated with blood transfusion ($P=0.002$, OR: 14.3 and CI: 2.5-104) and prison history ($P=0.01$, OR: 2.31 and CI: 1.2-4.49). Age, marital states, unsafe sex contact, and injection drug users were not associated with prevalence of HDV infection in HIV/HBV co-infected patients. According to the study findings, ALT and AST levels were not significantly different in patients infected with HDV compared with those negative for HDV.

Discussion

HIV, HBV, and HDV share the same routes of transmission. The presence of HDV infection among HIV/HBV co-infected patients has been associated with aggressive course of liver disease; frequently leading to cirrhosis, decompensation, and death.^{1,18} Anti-HDV antibody assay is highly recommended in HIV patients co-infected with HBV, particularly in those who live in endemic regions for HDV.

In the present study, the prevalence of HDV Ab among HIV/HBV co-infected patients was 19.8% (35 out of 178). Vaziri et al. reported that 31.57% of HIV infected individuals with positive HBsAg in Kermanshah (west of Iran) had HDV Ab in their serum.¹⁷ Furthermore, according to Aghasadeghi et al., the prevalence of HDV in

hemodialysis patients co-infected with HIV/HBV in Tehran was 2.5%.⁸ Although the outcome of our study differs from other Iranian reports, the small sample size of other studies may influence their results.

Up to now, two studies have reported the seroprevalence of HDV in HBsAg positive in Shiraz, southwest of Iran. The first study on the epidemiology of HDV infection from Iran (1989) shows that 13.9% of asymptomatic hepatitis B carriers in Shiraz were positive for anti-HDV Ab.¹⁹ In the other study conducted in 2008, Taghavi et al.²⁰ reported that anti-HDV Ab positivity rate was 9.7% in chronic hepatitis B patients over the age of 15, showing a decrease in its prevalence in comparison with previous studies. Reported studies from Tehran, show that 2.5-9% of HBsAg positive patients were infected with HDV.¹⁹ Moreover, the prevalence of HDV in HBV infected patients in southeast of Iran was 10.7% in Kerman, 17% in Sistan-Baluchistan province and in northern part of Iran, it was 0.03% in Qum and 2% in Babul.^{7,19} Recently, in a meta-analysis reported by Amini et al., 7.8% of HBsAg positive individuals of Iran were infected with HDV.¹⁹ These data show that; (i) HDV infection is more common in HBsAg positive patients in the south of Iran, including Fars province, than northern areas, (ii) the prevalence of HDV is more frequent in HIV/HBV co-infected patients than those only infected with HBV.

Data reported from other countries show various prevalence rates of HDV infection among individuals infected with HIV and HBV. The prevalence of HDV among Brazilian individuals infected with HIV and HBV was 1.2% (1 out of 86).²¹ Other reports from Brazil have shown that HDV prevalence of 9.4% and 2.7% in HIV/HBV co-infected cases.^{15,22} Sheng et al. reported 22.2% prevalence of HDV in HIV/HBV co-infected individuals in Taiwan.²³ In Italy, 11% (2 out of 18) of patients with dual infection of HIV and HBV were positive for HDV infection.²⁴ Although the prevalence of HDV in

Table 1: Main characteristics of HIV-infected individuals with positive HBs Ag according to delta status

| Variable | All HBsAg patients | HDV Ab-positive | HDV Ab-negative | P value |
|------------------------------|--------------------|-----------------|-----------------|---------|
| Number | 178 | 35 (19.7%) | 143 (80.3%) | - |
| Median age (years) | 34 (22-62) | 38 (30-62) | 37 (22-58) | 0.045 |
| Marital status | 29 | 6 (17%) | 23 (15.4%) | 0.9 |
| Unsafe sexual contact | 40 | 6 (17.1%) | 34 (23.7%) | 0.64 |
| Intravenous drug use | 68 | 15 (42.9%) | 53 (37%) | 0.8 |
| History of blood transfusion | 9 | 7 (20%) | 2 (1.4%) | 0.004 |
| History of being imprisoned | 69 | 25 (71.4%) | 44 (30.7%) | 0.01 |
| ALT level (IU/L) | 13.6 ± 8.8 | 13.2 ± 5.5 | 13.7 ± 9.4 | 0.95 |
| AST level (IU/L) | 9.2 ± 6.2 | 8.8 ± 3.7 | 9.3 ± 6.6 | 0.92 |
| Median CD4 count (cells/uL) | 281 | 276 | 289 | 0.47 |

Iran is low compared with other endemic regions (e.g. Turkey and Italy), comparison of data from Iran with other countries shows a high prevalence of HDV among Iranian patients with HIV/HBV co-infection. Moreover, HDV infection rate in the south of Iran is higher than that of the northern areas and HDV infection is more prevalent in patients infected with HIV than individuals with no history of HIV infection. In addition, HDV co-infection in patients infected with HIV and HBV may precipitate the progression of the disease and these patients may suffer from a more severe form of the disease than patients infected only with HIV and HBV. Accordingly, we recommend that screening for HDV infection should be mandatory in all HBsAg-positive/HIV-positive patients in Iran, particularly for those living in the areas with a high prevalence of HDV, such as Fars province, south of Iran.

HDV is spread mainly through parenteral exposure in the same way as HBV.²⁵ Our data showed that HDV infection in HIV/HBV co-infected patients was statistically significant and associated with blood transfusion and prison history but not with marital status, unsafe sexual contact, and intravenous drug use. In this study, the history of blood transfusion was statistically associated with HDV infection in HIV/HBV co-infected patients compared with those infected only with HBV (20% versus 1.4%). In another study in Isfahan, central Iran, HDV infection was more frequent in HBsAg positive patients with a history of transfusion.⁹ Therefore, finding the frequency of HDV in high-risk groups, including continual recipients of blood and blood products (e.g., patients with thalassemia, hemophilia, and coagulation factor deficiencies) is necessary in Shiraz and other parts of Iran. It has been reported that HDV marker positivity is associated with prison history.¹⁷ In line with these findings, our data also showed that the rate of HDV Ab in HIV/HBV co-infected individuals with prison history is higher than those without (71.4% versus 30.7%).

In this study, all individuals infected with HDV/HIV and HBV (35 out of 35) and 97.9% of those infected with HIV/HBV (140 out of 143) were male. This may be the reflection of higher prevalence of HIV in men than women in Iran.

HDV infection is more common in groups with frequent skin contact such as intravenous drug users. In a study conducted in Europe, the proportion of anti-HDV positive in HIV/HBV co-infected patients was higher in intravenous drug users.¹ Our data show that intravenous drug use was not a risk factor for HDV infection in HIV/HBV co-infected patients. With respect to the age as a risk factor for HDV infection, as

reported in some studies,¹ we did not find an association between age and HDV infection in HIV/HBV co-infected patients. Although sexual transmission has been reported as a possible route of HDV transmission, in the present study, marital status and unsafe sexual contact were not statistically associated with the high rate of HDV infection in HIV/HBV positive patients. Infection with HDV along with its genotyping can be detected by HDV RNA assay and this may be a limitation of our study. However, we have not performed such an assay on the studied HDV infected samples, as genotype detection was not included in this study.

Conclusion

Our study showed that HDV has affected a significant number of HIV infected population in Shiraz, southwest of Iran (19.7%). This emphasizes the necessity of HDV testing in this group of patients. According to the results, blood transfusion and prison history are considered as major risk factors of HDV infection in patients infected with HIV and HBV, but marital status and unsafe sexual contact are not. High infection rate of HDV Ab in the above-mentioned groups indicates that HDV transmission occurs more frequently in the parental route than a sexual route. It signifies the recommendation of blood screening for HDV diagnosis in thalassemia, hemophilia, and dialysis groups.

Acknowledgement

The authors would like to gratefully acknowledge all those participated in this study and the financial support from the Health Policy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Conflicts of Interest: None declared.

References

1. Soriano V, Grint D, d'Arminio Monforte A, Horban A, Leen C, Poveda E, et al. Hepatitis delta in HIV-infected individuals in Europe. *AIDS*. 2011;25:1987-92. doi: 10.1097/QAD.0b013e32834babb3. PubMed PMID: 21857493.
2. Kew MC. Hepatitis viruses (other than hepatitis B and C viruses) as causes of hepatocellular carcinoma: an update. *J Viral Hepat*. 2013;20:149-57. doi: 10.1111/jvh.12043. PubMed PMID: 23383653.
3. Sarvari J, Mojtahedi Z, Taghavi SA, Kuramitsu Y, Shamsi Shahrabadi M,

- Ghaderi A, et al. Differentially Expressed Proteins in Chronic Active Hepatitis, Cirrhosis, and HCC Related to HCV Infection in Comparison With HBV Infection: A proteomics study. *Hepat Mon.* 2013;13:e8351. doi: 10.5812/hepatmon.8351. PubMed PMID: 24066001; PubMed Central PMCID: PMC3776151.
4. Abbas Z, Jafri W, Raza S. Hepatitis D: Scenario in the Asia-Pacific region. *World J Gastroenterol.* 2010;16:554-62. PubMed PMID: 20128022; PubMed Central PMCID: PMC372816266.
 5. Amini N, Alavian SM, Kabir A, Aalaei-Andabili SH, Saiedi Hosseini SY, Rizzetto M. Prevalence of hepatitis d in the eastern mediterranean region: systematic review and meta analysis. *Hepat Mon.* 2013;13:e8210. doi: 10.5812/hepatmon.8210. PubMed PMID: 23554822; PubMed Central PMCID: PMC3609404.
 6. Attaran MS, Sharifi Z, Hosseini SM, Samei S, Ataee Z. Prevalence of hepatitis B and hepatitis D coinfection in asymptomatic blood donors in Iran. *APMIS.* 2014;122:243-7. doi: 10.1111/apm.12137. PubMed PMID: 23879563.
 7. 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:e6731. doi: 10.5812/hepatmon.6731. PubMed PMID: 24098308; PubMed Central PMCID: PMC3787685.
 8. Aghasadeghi 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:e7481. doi: 10.5812/hepatmon.7481. PubMed PMID: 23914228; PubMed Central PMCID: PMC3728973.
 9. Ataei B, Yazdani MR, Kalantari H, Yaran M, Nokhodian Z, Javadi AA, et al. Hepatitis D virus infection in Isfahan, central Iran: Prevalence and risk factors among chronic HBV infection cases. *Hepat Mon.* 2011;11:269-72. PubMed PMID: 22706272; PubMed Central PMCID: PMC3206699.
 10. Zahedi MJ, Darvish Moghaddam S, Alavian SM, Dalili M. Seroprevalence of Hepatitis Viruses B, C, D and HIV Infection Among Hemodialysis Patients in Kerman Province, South-East Iran. *Hepat Mon.* 2012;12:339-43. doi: 10.5812/hepatmon.5969. PubMed PMID: 22783346; PubMed Central PMCID: PMC3389360.
 11. Gozlan J, Lacombe K, Gault E, Raguin G, Girard PM. Complete cure of HBV-HDV co-infection after 24weeks of combination therapy with pegylated interferon and ribavirin in a patient co-infected with HBV/HCV/HDV/HIV. *J Hepatol.* 2009;50:432-4. doi: 10.1016/j.jhep.2008.05.029. PubMed PMID: 19070927.
 12. Puoti M, Airoldi M, Bruno R, Zanini B, Spinetti A, Pezzoli C, et al. Hepatitis B virus co-infection in human immunodeficiency virus-infected subjects. *AIDS Rev.* 2002;4:27-35. PubMed PMID: 11998781.
 13. Taylor JM. Hepatitis delta virus. *Virology.* 2006;344:71-6. doi: 10.1016/j.virol.2005.09.033. PubMed PMID: 16364738.
 14. Cross TJ, Rizzi P, Horner M, Jolly A, Hussain MJ, Smith HM, et al. The increasing prevalence of hepatitis delta virus (HDV) infection in South London. *J Med Virol.* 2008;80:277-82. doi: 10.1002/jmv.21078. PubMed PMID: 18098143.
 15. Soriano V, Vispo E, Labarga P, Medrano J, Barreiro P. Viral hepatitis and HIV co-infection. *Antiviral Res.* 2010;85:303-15. doi: 10.1016/j.antiviral.2009.10.021. PubMed PMID: 19887087.
 16. Ziaee MD, Azarkar G. Prevalence of hepatitis D virus infection among patients with chronic hepatitis B attending Birjand hepatitis clinic (East of Iran) in 2012. *Hepat Mon.* 2013;13:e11168. doi: 10.5812/hepatmon.11168. PubMed PMID: 24171009; PubMed Central PMCID: PMC3800676.
 17. Vaziri S, Mansouri F, Sayad B, Afsharian M, Janbakhsh A, Karami M. Hepatitis D Virus Infection among HIV-HBV Co-Infected Patients in Kermanshah, West of Iran. *Hepat Mon.* 2008;8:252-7.
 18. Calle Serrano B, Manns MP, Wedemeyer H. Hepatitis delta and HIV infection. *Semin Liver Dis.* 2012;32:120-9. doi: 10.1055/s-0032-1316467. PubMed PMID: 22760651.
 19. Amini N, Alavian SM, Kabir A, Saiedi Hosseini SY, Aalaei Andabili SH. Clinical Features and Seroepidemiology of Anti-HDV Antibody in patients With Chronic Hepatitis B Virus Infection in Iran: A Meta-Analysis. *Hepat Mon.* 2011;11:960-7. doi: 10.5812/kowsar.1735143X.805. PubMed PMID: 22368679; PubMed Central PMCID: PMC3282028.
 20. Taghavi SA, Sedighi S, Mehrabani D, Khademolhosesseini F. Hepatitis D in Chronic Active Hepatitis B: Prevalence, Liver Enzyme Levels and Histopathology- an Epidemiological Study in Shiraz, Southern

- Iran, 2003-2004. *Hepat Mon.* 2008;8:248-51.
21. Mendes-Correa MC, Gomes-Gouvea MS, Alvarado-Mora MV, Da Silva MH, Lazari C, Cavalcanti NC, et al. Hepatitis delta in HIV/HBV co-infected patients in Brazil: is it important? *Int J Infect Dis.* 2011;15:e828-32. doi: 10.1016/j.ijid.2011.08.003. PubMed PMID: 21999909.
 22. Braga WS, da Costa Castilho M, dos Santos IC, Moura MA, Segurado AC. Low prevalence of hepatitis B virus, hepatitis D virus and hepatitis C virus among patients with human immunodeficiency virus or acquired immunodeficiency syndrome in the Brazilian Amazon basin. *Revista da Sociedade Brasileira de Medicina Tropical.* 2006;39(6):519-22. Epub 2007/02/20. PubMed PMID: 17308694.
 23. Sheng WH, Hung CC, Kao JH, Chang SY, Chen MY, Hsieh SM, et al. Impact of hepatitis D virus infection on the long-term outcomes of patients with hepatitis B virus and HIV coinfection in the era of highly active antiretroviral therapy: a matched cohort study. *Clin Infect Dis.* 2007;44:988-95. doi: 10.1086/511867. PubMed PMID: 17342655.
 24. Morsica G, Bagaglio S, Cicconi P, Capobianchi MR, Pellizzer G, Caramello P, et al. Viral interference between hepatitis B, C, and D viruses in dual and triple infections in HIV-positive patients. *J Acquir Immune Defic Syndr.* 2009;51:574-81. doi: 10.1097/QAI.0b013e3181add592. PubMed PMID: 19590432.
 25. Wedemeyer H, Manns MP. Epidemiology, pathogenesis and management of hepatitis D: update and challenges ahead. *Nat Rev Gastroenterol Hepatol.* 2010;7(1):31-40. doi: 10.1038/nrgastro.2009.205. PubMed PMID: 20051970.

Archive of SID