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Changing Hepatitis D Virus Epidemiology in a Hepatitis B Virus Endemic Area With a National Vaccination Program

Hsi-Hsun Lin,[1,2] Susan Shin-Jung Lee,[3,4] Ming-Lung Yu,[5*] Ting-Tsung Chang,[6,7*] Chien-Wei Su,[1,3,8] Bor-Shen Hu,[9] Yaw-Sen Chen,[10] Chun-Kai Huang,[2] Chung-Hsu Lai,[2] Jiun-Nong Lin,[2] and Jaw-Ching Wu[1,11]

The emergence of hepatitis D virus (HDV) infection in the era of widespread HBV vaccination has not been described before. We aimed to investigate the changing epidemiology of HDV infection among high- and low-risk populations after an outbreak of human immunodeficiency virus (HIV) infection among injection drug users (IDUs) in Taiwan. A prospective, multicenter, cohort study of 2,562 hepatitis B surface antigen (HBsAg)-positive individuals was conducted to determine the prevalence, genotype, and risk factors of HDV infection from 2001 through 2012. The prevalence rates of HDV infection were 74.9%, 43.9%, 11.4%, 11.1%, and 4.4% among HIV-infected IDUs, HIV-uninfected IDUs, HIV-infected men who have sex with men, HIV-infected heterosexuals, and the general population of HBsAg-positive subjects, respectively. A significant increase in the trend of HDV prevalence from 38.5% to 89.8% was observed in HIV-infected IDUs (odds ratio = 3.06; 95% confidence interval: 1.68-5.56; $P = 0.0002$). In multivariate analysis, injection drug use, hepatitis C virus infection, HIV infection, serum HBsAg level ≧250 IU/mL, duration of drug use, and older age were significant factors associated with HDV infection. HDV genotype IV (72.2%) was the prevalent genotype circulating among IDUs, whereas genotype II was predominant in the non-IDU populations (73.3%). In the HIV cohort born after 1987 who were HBsAg negative, over half (52.9%) had antibody to hepatitis B surface antigen antibody levels of <10 mIU/mL and there was a significantly higher HBsAg seroprevalence in the HIV cohort, compared to the control group (8.1% vs. 0.0%; $P = 0.02$). *Conclusion*: In the era of HBV vaccination, IDUs and HIV-infected individuals have emerged as high-risk groups and a reservoir for HDV infection. Effective strategies are needed to curb the reemerging epidemic of HDV infection in these high-risk groups. (HEPATOLOGY 2015;61:1870-1879)

Hepatitis D virus (HDV) is a defective, single-stranded RNA virus that requires hepatitis B surface antigen (HBsAg) envelope for assembly and transmission.[1,2] Studies show that most patients with hepatitis B virus (HBV) and HDV dual infections have more severe liver disease, more rapid progression to cirrhosis, and increased frequency of hepatic decompensation and hepatocellular carcinoma

Abbreviations: Abs, antibodies; ALT, alanine transaminase; anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen; AST, aspartate transaminase; CAH, chronic active hepatitis; CHB, chronic hepatitis B; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; IDUs, injection drug users; LC, liver cirrhosis; MSM, men who have sex with men; OR, odds ratio; PCR, polymerase chain reaction.

From the [1]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Department of Medicine and Infection Control, E-Da Hospital/I-Shou University, Kaohsiung, Taiwan; [3]School of Medicine, National Yang-Ming University, Taipei, Taiwan; [4]Department of Internal Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; [5]Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; [6]Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan; [7]Department of Medicine, Medical College of National Cheng Kung University, Tainan, Taiwan; [8]Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; [9]Section of Infectious Diseases, Taipei City Hospital, Taipei City Government, Taipei, Taiwan; [10]Department of General Surgery, E-Da Hospital/I-Shou University, Kaohsiung, Taiwan; [11]Translational Research Division, Medical Research Department, Taipei Veterans General Hospital, Taipei, Taiwan

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**These authors made an equal contribution.*

(HCC).[3] Many studies report a poor response rate to interferon treatment and the ineffectiveness of nucleoside/nucleotide analog treatment.[4] Accurate estimation of updated prevalence and risk factors is important to identify risk groups to screen and make an effective policy to control the spread of HDV.

Approximately 15-20 million people are infected with HDV worldwide; however, its prevalence varies in different geographic regions.[4,5] In the past three decades, several longitudinal studies show that the prevalence of HDV is decreasing in formerly highly endemic areas, such as Italy, Spain, Turkey, and Taiwan.[6,7] However, HDV infection has recently reemerged with clustered outbreaks of HDV superinfection among high-risk populations in Venezuela, Ecuador, Mongolia, Greenland, Samara (Russia), Okinawa (Japan), Central Africa, and the Amazon basin, as well as in the immigrant population from endemic areas in Europe.[4]

Taiwan is an endemic area of HBV infection. Before the implementation of a nation-wide HBV vaccination program, the prevalence rate of HBV infection was 15%-20% in the general population. The vaccination program was launched in July 1984 to include newborns of high-risk, HBsAg-positive mothers and extended to all newborns after July 1986.[8] Thereafter, the rate of superinfection with HDV in patients with chronic hepatitis B (CHB) with acute exacerbations decreased from 23.7% in 1983 to 4.2% in 1995.[7] A similar decline in HDV prevalence of injection drug users (IDUs) and prostitutes in Taiwan was observed in 2002, falling to a rate of 14% and 5%, respectively.[9] Smaller studies among IDUs with and without human immunodeficiency virus (HIV) infection reported a varying prevalence of HDV infection from 10% to 91%.[10-16] This decline may be attributed to the successful implementation of the nation-wide HBV vaccination program,[8] as well as sustained educational efforts to the general public.

Between 2003 and 2006, an outbreak of HIV and hepatitis C virus (HCV) coinfection, originating from a geographically large transmission network from China, occurred among IDUs in Taiwan.[17,18] In this outbreak, our group reported an extremely high prevalence of HCV coinfection (up to 98%) and discovered the introduction of several novel HCV genotypes into Taiwan.[17] We hypothesized that this outbreak may have also led to a major change in the prevalence and genotype of HDV infections among IDUs and HIV-infected individuals in Taiwan. The identification of risk factors causing HDV infections in different populations is crucial for public health measures to control HDV infections. The current study aims to investigate the current prevalence, genotype, and risk factors causing HDV infections in various populations in Taiwan in an era of 30 years after a national HBV vaccination program.

Patients and Methods

Study Population. A multicenter, prospective, longitudinal, cohort study of HBsAg-positive individuals was conducted from 2001 through 2012. Six referral hospitals designated for hepatitis and HIV/acquired immune deficiency syndrome care in Taiwan participated in this study, including Taipei Veterans General Hospital (Taipei, Taiwan), Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan), Kaohsiung Medical University Hospital (Kaohsiung, Taiwan), National Cheng Kung University Hospital (Tainan, Taiwan), Taipei Municipal Venereal Disease Control Institute (Taipei, Taiwan), and E-Da Hospital (Kaohsiung, Taiwan). A total of 2,562 individuals were identified to be serologically positive for HBsAg, including 2,029 HBsAg-positive subjects who were followed up at outpatient clinics (304 diagnosed with HCC and 1,725 without HCC) from the general population, 369 individuals with HIV infection (263 IDUs, 70 men who have sex with men [MSM], and 36 heterosexuals), and 164 HIV-uninfected IDUs from the methadone outpatient clinic (Fig. 1). The HIV cohort of 369 HIV-infected individuals with serum HBsAg positivity was recruited from 1,662 HIV-infected individuals taken care of at the HIV outpatient clinic. The HIV-uninfected IDU cohort consisted of 164 of 218 HBsAg-positive individuals and was recruited from 1,157 IDUs attending the methadone outpatient clinics. The seroprevalence rate of HBsAg in the community was derived from a

Address reprint requests to: Jaw-Ching Wu, M.D., Ph.D., Translational Research Division, Medical Research Department, Taipei Veterans General Hospital and Institute of Clinical Medicine, National Yang-Ming University, 201 Shih-Pai Road, Section 2, Taipei 11217, Taiwan. E-mail: jcwu@vghtpe.gov.tw; fax: +886-2-2874-5074.

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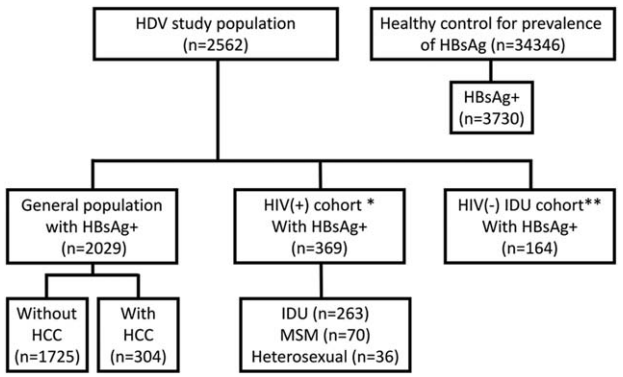

Fig. 1. Flowchart of study subjects who entered the study. *HIV(+) cohort (n = 1661); **HIV(−) IDU cohort (n = 1157); HDV: hepatitis delta virus; HBsAg: hepatitis B virus surface antigen; IDU: injection drug users; MSM: men who have sex with men.

control group of 34,346 healthy, non-IDUs and HIV-uninfected individuals undergoing routine health checkup during the study period.[19] The clinical status of the patients was defined as follows: (1) inactive carrier state: patients who are asymptomatic, have normal alanine transaminase (ALT) and aspartate transaminase (AST) levels, normal sonography, and either negative for hepatitis B e antigen (HBeAg) with an HBV-DNA load less than 2,000 IU/mL or HBeAg positive and HBV-DNA load <20,000 IU/mL; (2) chronic active hepatitis (CAH): patients who had either elevated ALT and AST levels without cirrhosis on sonography, or HBeAg positive with HBV DNA >20,000 IU/mL or HBeAg negative with HBV-DNA load >2,000 IU/mL; and (3) HBV-related cirrhosis: any marker of portal hypertension or ultrasonographic finding of small and coarse echogenicity of liver with round edges. Superinfection with HDV was defined as seroconversion of anti-HDV or low anti-HDV titer ≤100 dilution at acute exacerbation of hepatitis (ALT level ≧400 IU/L) in CHB carriers.[20]

Laboratory Test. Serum antibodies (Abs) to HCV and HIV, HBeAg, the antibody to hepatitis B core antigen (anti-HBc), HBsAg, and quantitative HBsAg levels were assessed by using the Abbott Architect system kits (Abbott Laboratories, Sligo, Ireland). Anti-HDV immunoglobulin G Ab was determined using the ANTI-HDV enzyme-linked immunosorbent assay kit (DiaSorin, Saluggia, Italy). Quantification of HBV DNA was tested using the Cobas TaqMan with a lower limit of detection of 6 IU/mL (Roche Diagnostics, Mannheim, Germany). Genotyping of HBV was performed by polymerase chain reaction (PCR) restriction fragment-length polymorphism of the surface gene of HBV.[21] Serum HDV RNA was detected using in-house real-time PCR, as previously described.[22] Sensitivity of the real-time PCR method assay to detect HDV RNA was 400 copies/mL and the linearity of quantification ranged from 2×10^3 to 2×10^9 copies/mL. HIV plasma viral load was determined using a Cobas Amplicor HIV-1 Monitor Test (version 1.5; Roche Diagnostics) or the HIV-1 RNA 3.0 Assay (bDNA; Siemens, Tarrytown, NY), according to the manufacturers' protocols. Demographic characteristics and behavioral information were collected during interviews. The study protocol was approved by the local institutional review boards.

HDV Genotype. To determine HDV genotype, viral RNA was extracted from 140 μL of plasma using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), and nested reverse-transcription PCR was performed to amplify the HDV delta-gene fragment (nt856-1275 relative to HDV reference strain JA-M27), as described elsewhere.[23] The first primer pairs used were HDV850 (5′-CGG ATG CCC AGG TCG GAC C-3′) and HDV1380 (5'-GGA GCW CCC CCG GCG AAG A-3′). The second primer pairs used were HDV-856 (5′-AGG TGG AGA TGC CAT GCC GAC-3′) and HDV-1275 (5′-GGA YCA CCG AAG AAG GAA GGC C-3′). After purification with a QIA quick PCR Purification kit (Qiagen), samples were screened with XhoI restriction fragment-length polymorphism analysis and then sequenced using an automatic sequencer (3100 Avent Genetic Analyzer, ABI; Applied Biosystems, Foster City, CA).[22] Phylogenetic analysis of a 419-base-pair fragment covering the HDV delta gene fragment was used to determine the HDV genotypes. Sequences were compiled using the BioEdit program (version 7.2.5; http://www.mbio.ncsu.edu/bioedit/bioedit.html), MEGA6 (molecular evolutionary genetics analysis, version 6.0), and CLUSTAL_X. To eliminate potential contamination, all of the sequences obtained were subjected to an HDV BLAST search to compare them with related reference sequences in the HDV database from the Gene Bank of the National Center for Biotechnology Information (Bethesda, MD). Genotypes were assigned after alignment with reference sequences. The following controls were used to construct a tree: HDV genotype I: X85253, X77627, M92448; HDV genotype II: TW2476, X60193; HDV genotype IIb-M: AF309420; HDV genotype III: AB037948; HDV genotype IV: AF209859, TWD62 (AF018077), AY452981; HDV genotype V: AM183326: HDV genotype VI: AM183329; HDV genotype VII: AM183333; and HDV genotype VIII: AX741169. The genetic distance of the HDV sequences analyzed was calculated using the two-parameter model used by Kimura. Phylogenetic

Table 1. Demographic Characteristics of the HBV Carriers in General Population and Various Risk Groups (n = 2,562)

		General Population of HBsAg (+)			HIV Negative	HIV-Positive Patients (n = 369)			
	Total	All	HCC(−)	HCC(+)	IDUs	IDUs	MSM	Heterosexual	
Characteristic	(n = 2,562)	(n = 2,029)	(n = 1,725)	(n = 304)	(n = 164)	(n = 263)	(n = 70)	(n = 36)	*P* Value
Age, years, mean, SD	46.6, 13.3	48.6, 13.7	47.2, 13.5	56.5, 12.3	39.5, 8.0	37.9, 7.2	38.0, 7.8	46.2, 12.4	<0.0001
(range)	(9.0-101.0)	(9.0-101.0)	(9.0-101.0)	(28.0-89.0)	(26.0-68.0)	(24.0-64.0)	(23.0-69.0)	(26.0-74.0)	
Sex, male, no. (%)	1,994 (77.8)	1,496 (73.7)	1,247 (72.3)	248 (81.6)	145 (88.4)	251 (95.4)	70 (100.0)	33 (91.7)	<0.001
AST >38 IU/L, no. (%)	1,273 (59.2)	1,055 (63.2)	908 (63.7)	146 (59.8)	63 (38.4)	127 (52.7)	21 (38.9)	8 (33.3)	<0.001
ALT >40 IU/L, no. (%)	1,528 (60.9)	1,279 (63.2)	1,118 (65.0)	160 (52.6)	77 (47.0)	137 (56.9)	25 (44.6)	11 (45.8)	<0.001
ALT ≥ 400 IU/L, no. (%)	246 (9.8)	243 (12.0)	230 (13.4)	13 (4.3)	0 (0.0)	2 (0.8)	0 (0.0)	1 (4.2)	<0.001
Inactive carrier, no. (%)	551 (21.5)	312 (15.4)			78 (47.6)	108 (41.4)	36 (51.4)	17 (47.2)	<0.001
CAH, no. (%)	1,525 (59.5)	1,245 (61.3)			80 (48.8)	150 (57.0)	34 (48.6)	16 (44.4)	<0.001
Cirrhosis, no. (%)	324 (12.6)	313 (15.4)	168 (9.7)	145 (47.7)	3 (1.8)	5 (1.9)	0 (0.0)	3 (8.3)	<0.001
HCV seropositivity, no. (%)	451 (19.3)	73 (4.0)	58 (3.8)	15 (5.3)	132 (81.5)	234 (98.3)	8 (11.4)	4 (11.1)	<0.001
HBsAg level ≧250 IU/mL	1,576 (74.7)	1,255 (76.3)	1,058 (76.5)	196 (75.7)	106 (66.3)	168 (71.5)	34 (68.0)	14 (60.9)	0.02
HBeAg	568 (33.7)	520 (34.8)	487 (37.4)	33 (17.3)	1 (25.0)	30 (22.1)	12 (35.3)	5 (25.0)	<0.001
HBV viral load >100,000 IU/mL	1,091 (49.5)	1,041 (53.4)	924 (54.7)	117 (44.7)	3 (21.4)	25 (14.9)	9 (19.2)	13 (50.0)	<0.001
HBV genotype									
B	730 (65.1)	694 (65.0)	632 (65.6)	62 (60.2)	0 (0.0)	12 (57.1)	14 (82.4)	10 (62.5)	0.33
C	386 (34.4)	370 (34.6)	328 (34.0)	41 (39.8)	0 (0.0)	8 (38.1)	3 (17.7)	6 (37.5)	
Others*	5 (0.4)	4 (0.4)	4 (0.4)	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)	0 (0.0)	

*Data were 3 of genotypes B and C recombination, 1 of genotype A, and 1 of genotype D.

trees were generated using the neighbor-joining method implemented in the CLUSTAL_X 1.81 program. The branch significance was analyzed by bootstrap with 1,000 replicates. The trees were printed using TreeView software (version 1.6.6). SIMPLOT and BOOTSCAN of the SIMPLOT 3.5.1 program (http://sray.med.som.jhmi.edu/SCRoftware/simplot/) were used to determine potential intergenotypic recombination.[24]

Statistical Analysis. Results were analyzed using Stata software (v10.0; StataCorp LP, College Station, TX). Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test, as appropriate. The chi-squared test for trend was used to analyze the trend of proportions; 95% confidence interval (CI) was calculated for proportions. Continuous variables were analyzed using the Student *t* test. All tests were two-tailed and a *P* value <0.05 was considered significant. Logistic regression was used to analyze the risk factors for acquiring HDV infection. All variables with $P < 0.10$ in the univariate analysis were considered for inclusion in the multivariate model. Forward selection, using the likelihood ratio test, was used to select the final multivariate model for risk factors for acquiring HDV infection.

Results

Demographic Characteristics of Study Participants. A total of 2,562 HBsAg-positive individuals were investigated in this study, and the demographic characteristics are shown in Table 1. There were 1,994 (77.8%) males and 568 (22.2%) females, with a mean age of 46.6 years (range, 9-101). Age, sex, clinical status (inactive carrier, CAH, and presence of liver cirrhosis [LC]), HCV seropositivity, HBsAg titer, HBeAg, and HBV viral load were significantly different between the study groups. Among HBsAg-positive individuals attending outpatient clinics from the general population, 312 of 2,029 (15.4%) were inactive carriers for HBV, 1,245 of 2,029 (61.3%) had CAH, 313 (15.4%) suffered from LC (of which 145 had HCC), and 159 had HCC without LC. HCV seroprevalence was highest (98.3%) among HIV-infected IDUs and lowest in the general population of HBsAg-positive subjects (4%). HCV prevalence among the general population of HBsAg-positive subjects living in northern and southern Taiwan differed significantly (9 of 818 [1.1%] vs. 64 of 1,010 [6.3%]; $P < 0.001$).

Comparison of HBV Seroprevalence Rates Between the Health Checkup Control Group, HIV, and HIV-Uninfected IDU Cohorts. The prevalence rates of HBsAg in the HIV cohort (22.2%; 369 of 1,662) and HIV-uninfected IDU cohort (18.8%; 218 of 1,157) were both significantly higher than the control group (10.9%; 3,730 of 34,346; $P < 0.001$). The seroprevalence of antibody to hepatitis B surface antigen (anti-HBs), anti-HBc, and isolated anti-HBc among the HIV cohort and HIV-uninfected IDUs was 57.9%, 82.5%, and 19.8% and 60.6%, 85%, and 20.9%, respectively. For individuals born after 1987, there was a significantly higher HBsAg seroprevalence in the HIV cohort, compared to the control group (8.1% [3 of 37] vs. 0% [0 of 97]; $P = 0.02$). In the

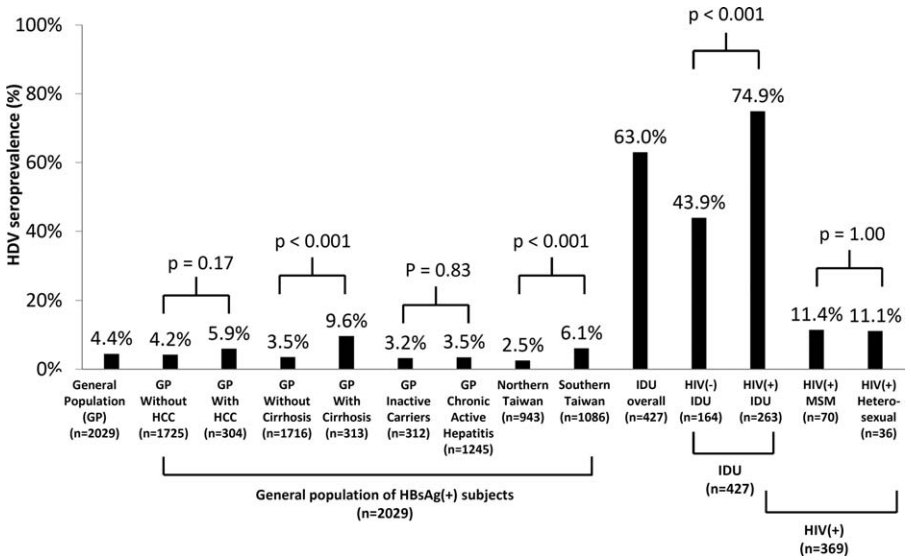

Fig. 2. Seroprevalence of HDV infection among various groups (n = 2,562).

HIV cohort born after 1987 who were HBsAg negative, over half (52.9%) had anti-HBs levels of <10 mIU/mL.

Prevalence of HDV Infection in Risk Groups and HBsAg-Positive Subjects From the General Population. The overall prevalence of HDV infection in HBsAg-positive individuals was 14.5% (371 of 2,562). However, there were distinct differences in prevalence rates among the different groups. The seroprevalence rates of HDV were 74.9%, 43.9%, 11.4%, 11.1%, and 4.4% among the HIV-infected IDUs, HIV-uninfected IDUs, HIV-infected MSM, HIV-infected heterosexuals, and HBsAg-positive subjects attending outpatient clinics from the general population, respectively (Fig. 2). The overall HDV prevalence among IDUs was 63% and was higher in HIV-infected IDUs than non-HIV-infected IDUs (74.9% vs. 43.9%; $P < 0.001$). HIV-infected IDUs had the highest risk for HDV infection (adjusted odds ratio [OR] = 76.61; 95% CI: 28.78-231.45). Among HBsAg-positive subjects attending outpatient clinics from the general population, HDV prevalence rates were 3.2%, 3.4%, 5.9%, and 9.6% among inactive carriers, CAH, HCC, and LC, respectively. A higher HDV prevalence was observed in those who had LC, compared to those without LC (9.6% vs. 3.5%; $P < 0.001$) and in those subjects living in southern Taiwan, compared to those in northern Taiwan (6.1% vs. 2.5%; $P < 0.001$).

Secular Change of HDV Prevalence in Different Subgroups. The trend of HDV prevalence in HIV-infected IDUs revealed a significant increase between 2001 and 2008 from 38.5% in the period 2001-2004 to 89.8% in 2009-2012 (OR = 3.06; 95% CI: 1.68-5.56; $P = 0.0002$, by the chi-squared test for trend; Fig. 3). No differences were observed in the other groups.

Incidence of Acute HDV Superinfection in the HBsAg-Positive Subjects From the General Population. Among HBsAg-positive subjects with acute exacerbations with an ALT level ≧400 IU/L from the general population who showed seroconversion of anti-HDV or low anti-HDV titer ≤100 dilution at acute exacerbation of hepatitis, defined as HDV superinfection, was 3.4% (8 of 237). This demonstrated a significant decrease in the incidence of HDV superinfection, compared with previous studies, reporting incidence rates of 14.6% (77 of 527; $P < 0.001$) in 1997 and 15% (9 of 60; $P = 0.002$) in 1999.[7,25] When analysis was stratified by HBeAg, the rate of HDV superinfection was 1.6% (2 of 126) for HBeAg-positive and 5.4% (6 of 111) for HBeAg-negative individuals.

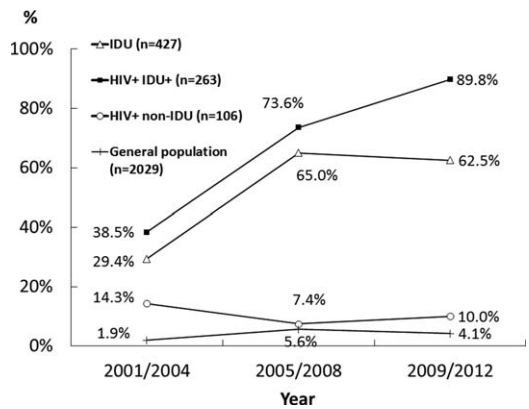

Fig. 3. Trend of HDV seroprevalence among different HBsAg-positive groups (n = 2,562) by 4-year period revealed a significant increase in HIV-infected IDUs from 38.5% in 2001-2004 to 89.8% in 2009-2012 (OR = 3.06; 95% CI: 1.68-5.56; $P = 0.0002$, by the chi-square test for trend) and no differences among the other groups.

Table 2. Multivariate Logistic Regression Analysis for the Risk Factors of HDV Infection (n = 2,562)

Factor	Crude OR	(95% CI)	*P* Value	Adjusted OR*	(95%CI)	*P* Value
Age, years						
<40	1.00			1.00		
40-49	0.54	(0.41-0.71)	<0.001	0.93	(0.64-1.37)	0.73
≥50	0.33	(0.25-0.44)	<0.001	1.62	(1.09-2.41)	0.02
Sex, male	2.89	(2.03-4.11)	<0.001			
HIV infection	16.38	(12.6-21.3)	<0.001	2.81	(1.94-4.05)	<0.001
HCV infection	25.16	(19.0-33.3)	<0.001	3.84	(2.34-6.31)	<0.001
HBsAg titer ≧250 IU/mL	1.26	(0.95-1.67)	0.11	2.39	(1.63-3.51)	<0.001
HBeAg positivity	0.49	(0.34-0.71)	<0.001			
HBV genotype						
B	1.00					
C	0.71	(0.37-1.37)	0.31			
AST >38 IU/L	0.78	(0.62-0.99)	0.04			
ALT >40 IU/L	0.86	(0.69-1.09)	0.21			
Injection drug use	33.93	(25.66-44.88)	<0.001	7.18	(4.25-12.14)	<0.001
Duration of drug use, years (n = 232)						
<5	1.00			1.00		
5-9	2.09	(1.04-4.18)	0.04	2.00	(0.92-4.39)	0.08
≧10	2.09	(1.11-3.92)	0.02	2.31	(1.11-4.80)	0.03

*The final multivariate model included age, HIV infection, HCV seropositivity, and IDU status.

Multivariate Analysis of Risk Factors Associated With HDV Infection. In multivariate logistic regression analysis adjusted for age, HIV infection, HCV seropositivity, and IDU status, major risk factors associated with HDV infection were injection drug use, HCV infection, HIV infection, serum HBsAg level ≧250 IU/mL, duration of drug use ≥10 years, and age ≥50 years (Table 2). There was a significantly increasing trend in the HDV prevalence with age in the non-IDU population (OR = 1.32; 95% CI: 1.09-1.60; $P = 0.005$, by the chi-squared test for trend; Fig. 4), but not among the IDUs (OR = 0.85; 95% CI: 0.67-1.08; $P = 0.18$). A significantly increasing trend in the cumulative HDV prevalence was observed in 232 IDUs with each year of injection drug use, in those using drugs for 15 years and less (OR = 1.14; 95% CI: 1.05-1.23; $P = 0.001$; Fig. 5).

Comparison of Patients With and Without HDV Viremia. HDV RNA was detectable in 148 of 342 samples from the anti-HDV-positive individuals (43.3%). Age, gender, transmission routes, HCV seropositive rate, and HIV viral loads were not different between patients with and without HDV viremia (Table 3). HBV viral factors, serum HBV-DNA levels, and HBeAg status were also similar between these two groups. However, HDV viremic individuals had a higher frequency of elevated liver transaminase levels and HBsAg levels of ≧250 IU/mL.

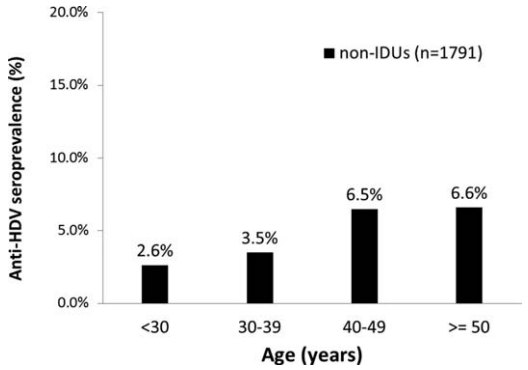

Fig. 4. Trend of HDV prevalence associated with age in the non-IDU population revealed a significant increase associated with age in the non-IDU population (n = 1,791; OR = 1.32; 95% CI: 1.09-1.60; $P = 0.005$, by the chi-squared test for trend).

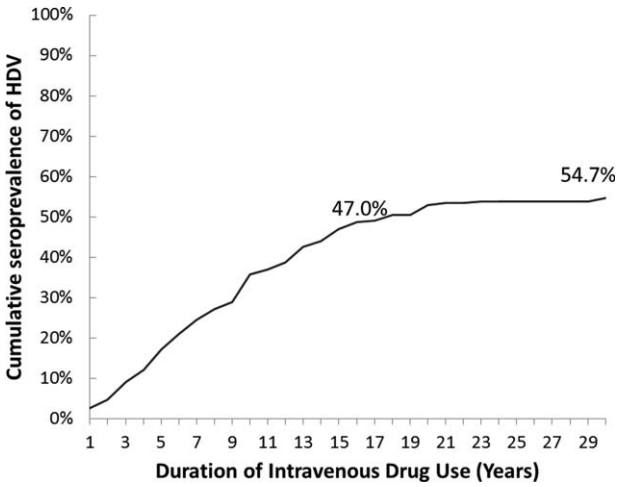

Fig. 5. Cumulative prevalence of HDV infection among the 232 IDUs revealed a significant increase associated with increasing duration of injection drug use (OR = 1.14; 95% CI: 1.05-1.23; $P = 0.001$).

Table 3. Basic Characteristics of the Patients With HDV Seropositivity, With and Without Detectable HDV RNA (n = 342)

Characteristic	N	HDV Seropositive (n = 342)	HDV-RNA Positive (n = 148)	HDV-RNA Negative (n = 194)	*P* Value
Age (mean, SD, range)	342	40, 10.5 (16.7-80.8)	38.3 , 9.6 (16.7-66.0)	41.4 , 10.8 (21.9-80.8)	0.20
Sex, male, n (%)	342	317 (92.7)	135 (91.2)	182 (93.8)	0.36
Risk factor for HIV infection	182	8 (4.1)	5 (6.1)	3 (2.6)	0.51
MSM		8 (4.1)	5 (6.1)	3 (2.6)	0.51
Heterosexual		4 (2.0)	2 (2.4)	2 (1.8)	
IDU		184 (93.9)	75 (91.5)	109 (95.6)	
CD4 cell count, cells/mm^3	141				
<200		5 (3.6)	2 (3.3)	3 (3.8)	0.95
200-349		34 (24.1)	14 (23.0)	20 (25.0)	
≥350		102 (72.3)	45 (73.7)	57 (71.2)	
HIV viral load, copies/mL	143				
<10,000		114 (79.7)	50 (82.0)	64 (78.1)	0.44
10,000-99,999		24 (16.8)	8 (13.1)	16 (19.5)	
≥100,000		5 (3.5)	3 (4.9)	2 (2.4)	
HCV seropositivity	305	217 (71.1)	92 (68.7)	125 (73.1)	0.40
Liver function tests	328				
AST >38 IU/L		167 (50.9)	79 (44.8)	88 (47.6)	0.18
ALT >40 IU/L		213 (64.9)	104 (72.7)	109 (58.9)	0.009
AST, IU/L, median (IQR) (range)		43 (31-68) (14-2010)	47 (33-77) (14-1660)	40 (29-65) (18-2010)	0.02
ALT, IU/L, median (IQR) (range)		53 (33-91) (11-1850)	57 (35-99) (12-1850)	47 (31-83) (11-953)	0.03
HBeAg positivity	161	25 (15.5)	10 (14.3)	15 (16.5)	0.70
HBsAg titer ≧250 IU/mL	304	234 (77.0)	118 (87.4)	116 (68.6)	<0.001
Serum HBV-DNA positive (%)	342	228 (66.7)	102 (68.9)	126 (65.0)	0.44
Median HBV DNA, IU/mL	228	441.5 (24-46,969)	619.5 (32-29,258)	357 (19-60,697)	0.54
HBV DNA ≧10^4		71 (31.1)	30 (29.4)	41 (32.5)	0.61

Distribution of HDV Genotypes in Risk Groups and HBsAg-Positive Subjects From the General Population. HDV genotypes were determined in 153 of 342 samples from HDV-positive individuals. Distribution of genotypes based on phylogenetic analysis is shown in Fig. 6 and summarized in Table 4. The main circulating HDV genotypes in our study were genotype IV (56.6%), genotype II (34.9%), and genotype I (8.6%). Genotype IV was the major prevalent HDV genotype circulating among the IDUs ($P < 0.001$), even when stratified by HIV status (Table 4). The main HDV genotype circulating in non-IDUs was genotype II. HDV genotype mix or recombination was not detected.

Discussion

Our study showed that there were distinct differences in the prevalence of HDV infection among different populations in an HBV endemic area, in the era of 30 years after a national HBV vaccination program. The prevalence of HDV infection among the HBsAg-positive subjects from the general population remained low in this study (4.4%); however, there was a significant decrease in the incidence of acute HDV superinfection in the general population.[7,25] In contrast, the burden of HDV in high-risk populations was exceptionally high. We demonstrated an extremely high prevalence of HDV infection among IDUs, particularly in those with HIV infection. A significantly increasing trend in the prevalence of HDV infection in HIV-infected IDUs was found from 2001 to 2012. This increasing trend may be explained by a higher prevalence of HBV infection in this population and the consequence of an explosive outbreak of HIV and HCV infection occurring in Taiwan just before the conduction of the study.[17,18]

IDUs who shares needles have the highest prevalence of HDV infection worldwide, with rates varying from 8% to more than 90%.[4,26] Our study revealed that injection drug use was a major risk factor for HDV infection, and that the cumulative HDV seroprevalence increased significantly with increasing years of injection drug use in those who had been users for 15 years or less. The association between the duration of injection drug use and prevalence of hepatitis B and C and HIV infection has been described.[27] However, there is scant literature on the association between HDV infection and the duration of injection drug use. Our study is the first to show a significant, positive trend in the cumulative HDV seroprevalence per year of injection drug use.

HCV infection is the second-strongest risk factor for HDV infection in our study, because it shares the same route of transmission as HDV. Likewise, many studies have reported an association between HDV

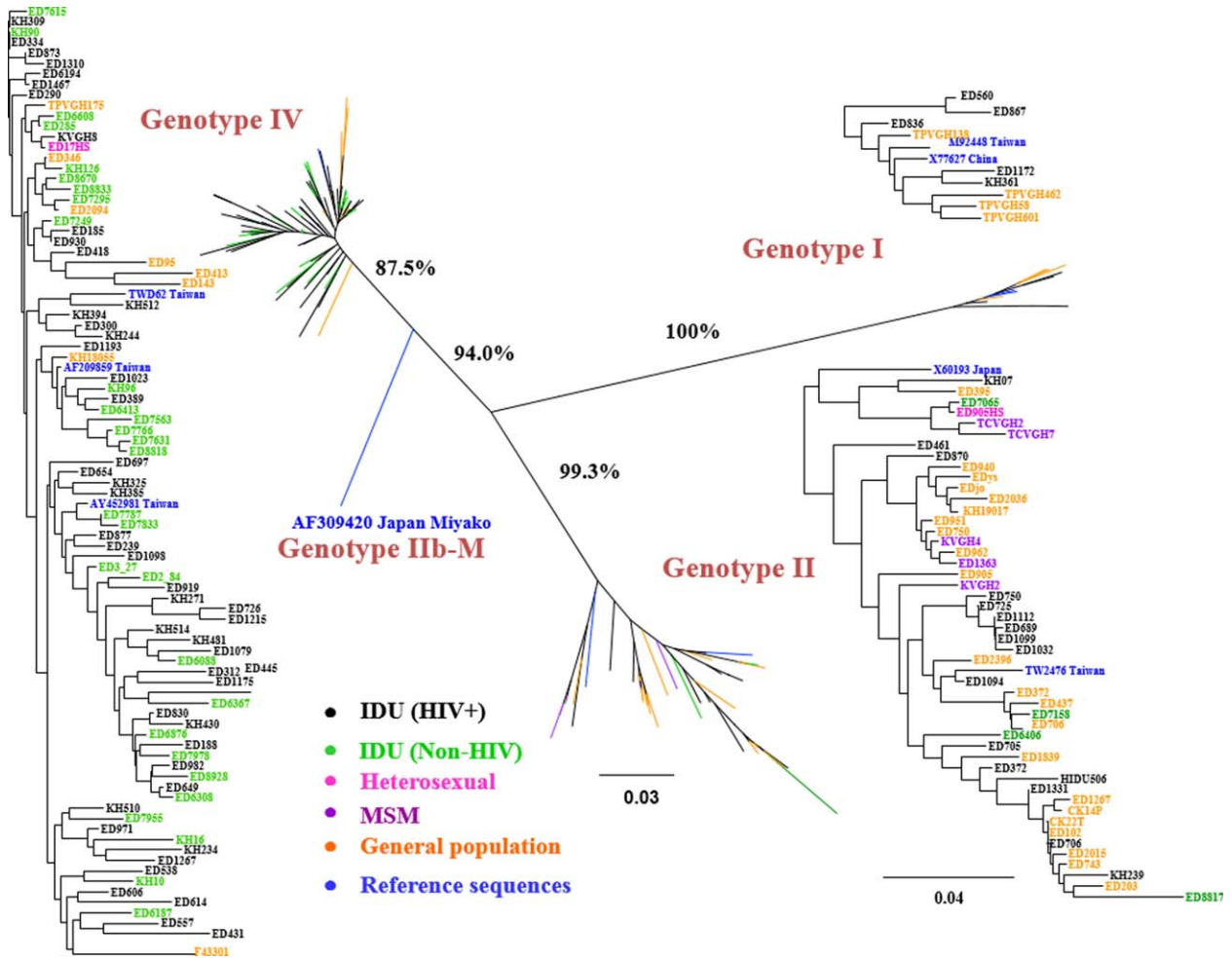

Fig. 6. Phylogenetic analysis based on HDV delta-gene sequences from the study population (n = 153). The horizontal branch was drawn in accord with the relative genetic distance. A number of commonly used reference delta-gene sequences for classifying HDV genotypes were also included and are indicated by accession numbers.

and HCV seropositivity. A large cohort study conducted in Central Europe showed that HCV coinfection is a frequent phenomenon, and approximately one third of patients with HDV infection tested positive for anti-HCV.[28] In a recent study from Northern California, approximately half of the HDV-infected individuals were also HCV infected.[29] Our study also showed that the geographical variation in the prevalence of HDV infection among the HBsAg-positive subjects from the general population paralleled the epidemiology of HCV infection, with a higher prevalence of HDV (6.1% vs. 2.5%; $P < 0.001$) and HCV (6.3% vs. 1.1%; $P < 0.001$) in southern Taiwan, compared to those in northern Taiwan. Geographic difference of HCV prevalence between southern and northern Taiwan has been reported before, and iatrogenic medical injections with reused, contaminated syringes were found to be the major risk factor.[15,30]

Table 4. Distribution of HDV Genotypes According to Risk Factors (n = 153)

Risk Factors (N = 153)	HDV Genotype, n (%)			*P* Value
	I	II	IV	
IDU vs. non-IDU				
Non-IDU (n = 45)	4 (8.9)	33 (73.3)	8 (17.8)	<0.001
IDU (n = 108)	9 (8.3)	21 (19.4)	78 (72.2)	
HIV infected vs. uninfected				
HIV uninfected (n = 70)	4 (5.7)	32 (45.7)	34 (48.6)	0.04
HIV infected (n = 83)	9 (10.8)	22 (26.5)	52 (62.7)	
IDU vs. non-IDU stratified by HIV status				
HIV positive (n = 83)				
IDUs (n = 76)	9 (11.8)	16 (21.1)	51 (67.1)	0.004
non-IDUs (n = 7)	0 (0.00)	6 (85.7)	1 (14.3)	
HIV negative (n = 70)				
IDU (n = 32)	0 (0.00)	5 (15.6)	27 (84.4)	<0.001
non-IDU (n = 38)	4 (10.5)	27 (71.1)	7 (18.4)	

Older age (≥50 years) was demonstrated to be a significant risk factor for HDV infection, and a significant increasing trend of HDV prevalence was found with age in the non-IDU group. This was consistent with previous studies.[31,32] Among non-IDUs, HIV-infected individuals, both MSM and heterosexuals, had a higher prevalence of HDV infection than the HBsAg-positive subjects from the general population. This finding suggested that people with high-risk sexual behavior are at an increased risk for HDV infection. HDV prevalence in HIV-infected persons did not differ significantly between MSM and heterosexuals in our study.

Importantly, our study found a higher HBV carrier rate in the vaccinated, HIV cohort, compared to the general population, born after nation-wide HBV vaccination. We also found that anti-HBs levels were below the level of protection in more than half of HIV and IDU cohorts who were not HBV carriers. The increased risk of acquiring HBV and HDV infection as well as the high rate of chronicity were most likely owing to both their immunocompromised status and the high-risk behavior leading to repeated exposures to HDV. This high-risk group may become a reservoir for HBV and HDV. Therefore, we suggest that an HBV vaccination booster may be indicated for HIV-infected persons who are HBsAg negative with low levels of anti-HBs (<10 mIU/mL), even if they had received HBV vaccination at birth. This is concordant with the recommendation for booster vaccination in immunocompromized patients by the European Consensus Group on Hepatitis B Immunity.[33] However, vaccine efficacy in HIV-infected individuals requires further study.

There are varied geographical distribution of different HDV genotypes[4]; however, whether the distribution of HDV genotype varies by risk group remains uncertain. The distribution of HIV and HCV genotypes has been reported to vary both geographically and by risk group.[34] In this study, we demonstrated that the HDV genotype circulating among the IDUs was distinct from those circulating among the HBsAg-positive subjects from the general population. Three genotypes of HDV have been reported in Taiwan previously, with a predominance of genotype II, varying from 85.4% in 1995, 82.8% in 1998, to 55.6% in 2006,[35-37] whereas genotype IV (genotype IIb in the old nomenclature) accounted for only 8.6% in 1998 and 13.1% in 2006. A small-scale study of 31 IDUs with HDV infection conducted in 2002 found a predominance of genotype II (58.0%) and genotype I (35.5%), but did not find any cases with genotype IV.[10] A more recent study describing the HDV genotypes among IDUs showed that genotypes II and IV were the two major genotypes.[12] Our study further demonstrated the changing molecular epidemiology of HDV infection in Taiwan, with a shift in the main circulating HDV genotypes to genotype IV (56.6%), followed by genotype II (34.9%) and genotype I (8.6%). Genotype IV was the major prevalent HDV genotype circulating among IDUs, even when stratified by HIV status. However, the main HDV genotype circulating in non-IDUs was genotype II.

Another interesting and novel finding in this study is the association of HDV infection and viremia with a serum HBsAg level ≧250 IU/mL. A cut-off value of 250 IU/mL was arbitrarily used because this value is the upper limit of the quantitative test used in our routine clinical practice. Recently, quantitative HBsAg has been used as a new maker to monitor the natural history and complement HBV-DNA levels to optimize the management of CHB patients.[38] A large, central European, cohort study revealed that the mean HBsAg levels did not differ significantly between HBV-monoinfected patients and individuals with delta hepatitis.[28] Our previous study revealed that the secretion of genotypes I, II, or IV generally correlated with HBsAg levels, but not with HBV genotypes or HBV-DNA levels.[23] The finding that high HBsAg levels was a risk factor for HDV infection and HDV-RNA viremia in patients with and without HIV coinfection can be explained by the fact that the assembly of HDV requires only HBsAg, and not HBV DNA.[1,2] It also implies that the suppression of HBsAg levels may be helpful in controlling HDV infection and viremia. An international study reported that serum levels of HBsAg showed a weak correlation with the histological activity of disease in patients with HDV infection.[39] However, further studies are needed to elucidate the role of serum levels of HBsAg in HDV infection.

In summary, IDUs, especially HIV-infected IDUs, have become the most important risk group in HDV infection and a reservoir for HDV, even after the implementation of a nation-wide HBV vaccination program for 30 years. The dominant HDV genotype in IDUs is genotype IV, in contrast to genotype II in the general population. Effective strategies, such as methadone maintenance therapy and clean syringe exchange programs, and new policies are needed to prevent injection drug use and educate IDUs on the avoidance of practices that may lead to infection with HDV.

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