Microbiol. Biotechnol. Lett. (2022), 50(3), 422–429
http://dx.doi.org/10.48022/mbl.2205.05003
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Analysis of Hepatitis C Virus Genotypes and RNA Quantitative Values in Cheonan, Korea from 2007 to 2016

Bishguurmaa Renchindorj[1†], Bo Kyeung Jung[2†], and Joowon Park[2*]

[1]*Department of Laboratory Medicine, Graduate School, Dankook University, Cheonan 31116, Republic of Korea*
[2]*Department of Laboratory Medicine, College of Medicine, Dankook University, Cheonan 31116, Republic of Korea*

Received: May 9, 2022 / Revised: July 21, 2022 / Accepted: July 22, 2022

The hepatitis C virus (HCV) genome contains a positive-sense single-stranded RNA molecule, and it is classified into 8 genotypes and 87 subtypes. Globally, over 350,000 people die from liver cirrhosis and hepatocellular carcinoma caused by HCV each year. Here, the genotype distribution of HCV was estimated in the population in Cheonan, Korea using Sanger sequencing. In addition, the correlation between HCV RNA level and genotype was assessed using real-time polymerase chain reaction (PCR); similarly, the correlation of HCV RNA level with isolation year (2007-2016) was determined using 463 consecutive serum samples obtained from patients at Dankook University Hospital, Cheonan, Korea. In 2007, genotype 1b (54.2%) was predominant, followed by genotypes 2a (41.7%), 1a (2.1%) and 3a (2.1%); whereas in 2016, the predominant genotype was 2a (49.0%), followed by genotypes 1b (46.9%), 3b (2%), and 4a (2%). Neither age nor sex was correlated with HCV genotype. Furthermore, the mean HCV RNA level decreased significantly from 2012 to 2016 ($p < 0.05$). However, no significant correlations between genotype and HCV RNA level were found. Overall, the findings revealed that genotypes 2a and 1b were the most common in Cheonan, and the prevalence of HCV genotype 1b tended to decrease over the past decade.

Keywords: **Hepatitis C virus, genotype, RNA quantitative value, Korea**

Introduction

The World Health Organization (WHO) reported that globally, 1.75 million people are infected with the hepatitis C virus (HCV) every year, and about 71 million people were living with chronic HCV infection in 2015. Globally, the prevalence of HCV infection is 1.0%, with the highest rates in the Mediterranean region (2.3%), followed by Europe (1.5%), and South Asia (0.5%) [1]. In 2009, the average seroprevalence of HCV infection in Korean population was estimated to be 0.78% (95% confidence interval, 0.71–0.81), as determined using the anti-HCV antibody assay. However, high seroprevalence rates of 1.53% and 2.07% were reported in Busan City and Jeolla Province in South Korea, respectively. The area with the lowest prevalence was Jesu Island (0.23%) [2].

The genome of HCV, a member of the genus *Hepacivirus*, family Flaviviridae, contains a 9.7–11.8-kb positive-sense single-strand RNA molecule [3, 4]. Globally, HCV has been classified into 8 genotypes and 87 subtypes [5, 6], with the genotypes varying geographically [7]. Approximately 83.4 million people have been diagnosed with HCV genotype 1 worldwide, accounting for 46.2% of all cases, with most of these patients living in East Asia. Genotype 3 is the second most common genotype worldwide (54.3 million cases, 30.1%), with almost all cases detected in South Asia. Genotypes 2 and 4 have been

***Corresponding author**
Phone: +82-41-550-3097, Fax: +82-41-550-7055
E-mail: joowon@dankook.ac.kr
†These authors contributed equally to this work.

detected in ~16.5 million (9.1%) and 15.0 million (8.3%) patients, respectively, with the latter being mostly distributed in North Africa and the Middle East [8]. Genotype 6 has been detected in 9.8 million (5.4%) patients. In Korea, liver cancer was reportedly the sixth most frequent cancer, ranking second in cancer-related mortality in 2014. Furthermore, HCV infection was identified in 10% of patients with hepatocellular carcinoma (HCC) in Korea in 2010s [9], with HCV genotype 1b associated with a higher risk of HCC than genotypes 2a, 2c, or other genotypes worldwide [10–12]. In Korea, the most frequently detected genotypes are genotypes 1b and 2a; however, the related studies are limited [2, 13, 14].

In 2016, major public health concerns forced the World Health Assembly to endorse a global strategy for eliminating HCV by 2030. Additionally, the WHO set targets for the successful treatment of 80% of eligible persons with chronic HCV infection, and to increase the rate of diagnostic screening to 90% by 2030 [1, 15]. In contrast to hepatitis B virus infection, there are currently no vaccines available to prevent HCV infection, and improved treatment strategies are urgently needed. Some recent drugs, including HCV direct-acting antivirals (DAAs), have been developed for the treatment of hepatitis C [16]; they were first approved by the US Food and Drug Admnistration in 2011, and the Korean regulatory authorities approved them in 2015 [17]. The duration of DAA therapy depends on the presence of cirrhosis, HCV genotype, and previous treatment failure [16]; however, limited knowledge regarding the prevalence of HCV genotypes in Korea reduces the efficacy of therapies.

Accordingly, in this study, the genotype distribution of HCV was estimated in the population in Cheonan, Korea from 2007 to 2016, and the findings were compared with those from other areas of Korea and countries. Furthermore, the correlations among the HCV RNA level, HCV genotypes, and isolation years were evaluated.

Materials and Methods

Sample collection

Between 2007 and 2016, serum samples were collected from 591 patients attending Dankook University Hospital, Cheonan, Korea. Samples from 128 patients were excluded owing to poor detection of the target sequence; thus, samples from 463 patients were included, in which HCV RNA was detected and analyzed for genotype. As all data were collected retrospectively using medical records, informed consent from patients was not required. The correlation of sex, age group, HCV RNA level, and isolation year with genotypes was investigated. The study was ethically approved by the institutional ethics committee of Dankook University Hospital (Approval no. 2020-08-024).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

HCV real-time PCR proceeds with PCR amplification and detection steps based on fully automated sample pre-processing (nucleic acid extraction and purification) using COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HCV Quantitative Test, v2.0 (part no. 05532264 190 Roche Diagnostics, Basel, Switzerland) on COBAS p 480 analyzer (Roche Diagnostics). Reverse transcription, amplification, and HCV RNA quantitation were implemented using HCV Master Mix reagent in CAP/CTM HCV test kit including Z05 and Z05D DNA polymerase, a primer pair to the 5' UTR region of HCV, and fluorescent-labeled oligonucleotide probes specific for HCV and the HCV Quantitation Standard (QS). The amplified RNA was detected based on an HCV-specific gene and a QS-specific probe; thus, the amplification products of HCV and QS were detected independently. HCV viral RNA was quantified based on QS, where a specific amount of HCV QS was mixed with each sample and a control, before being subjected to sample extraction, reverse transcription, PCR amplification, and detection with HCV RNA. The analyzer calculated the HCV RNA level by comparing the HCV and QS signals obtained from each sample and the control, leading to the accurate quantification of HCV RNA. Furthermore, the analyzer allowed for HCV targets to be simultaneously detected, identified, and the QS amplified in human plasma or serum.

HCV sequencing

RNA extraction was performed with 300 μl of serum contained in the serum-seperating tube (SST) using QIAamp MinElute virus spin kit (Qiagen, Germany) on QIAsymphony extraction equipment (Qiagen). After extraction, 8 μl of RNA was reverse transcribed to

cDNA, and cDNA was synthesized using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics). RT-PCR and sequencing were performed using HCV genotyping Kit (BioSewoom Inc., Korea) as follows according to the manufacturer's instructions. After combining 13 μl of PCR mixture, 2 μl of primer mixture, and 5 μl of distilled water in a PCR tube, 5 μl of DNA was added, and PCR was performed using MJ Research PTC-200 Thermal Cycler (Bio-Rad Laboratories, USA) under the following conditions: 95℃ 1 cycle for 10 min; 50 cycles at 94℃ for 30 s, 54℃ for 30 s, and 72℃ for 1 min; and 72℃ 1 cycle for 5 min. Following the reaction, PCR products were subjected to electrophoresis, and the results were analyzed based on the PCR band size (core gene: 408 bp, 5′ untranslated region: 293 bp). Purification was performed to remove dNTPs and primer dimers remaining in the sample. Subsequently, 1 μl of the purified PCR product was placed in a PCR tube, 9 μl of the sequencing mixture was added, and a sequencing reaction was performed using ABI genetic analyzer (Applied Biosystems, USA). The HCV genotype was identified using the LANL HIV database (http://hcv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html).

Statistical analysis

HCV genotype frequencies were estimated using chi-square tests; whereas means of HCV RNA levels were compared using *t*-tests (α = 0.05). All statistical analyses were performed using SPSS *v*.26.

Results

Characteristics of HCV genotypes

Between 2007 and 2016, 463 patients underwent testing for HCV RNA quantification and HCV genotype identification at Dankook University Hospital. The HCV genotype 2a was the most common (n = 234, 50.5%), followed by genotypes 1b (n = 209, 45.1%), 3a (n = 7, 1.5%), 1a (n = 5, 1.1%), 2b (n = 3, 0.6%), 4a (n = 2, 0.4%), 6a (n = 1, 0.2%), 1b+2a (n = 1, 0.2%), and 3b (n = 1, 0.2%; Table 1).

The prevalence of HCV infection in men (55.1%; 255/463) was higher than that in women (44.9%; 208/463), with an overall male-to-female ratio of 1.2:1; however, the difference was not statistically significant ($p > 0.05$; Table 1). The distribution of HCV genotypes by sex is delineated in Fig. 1.

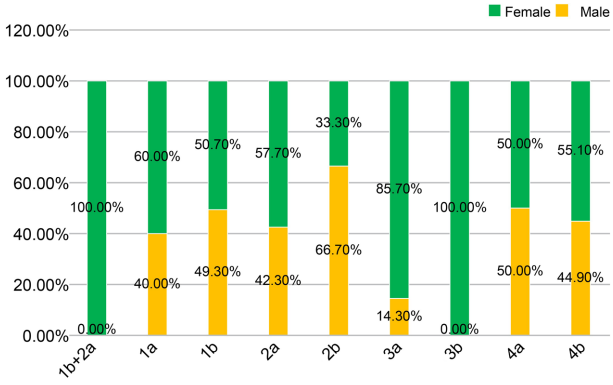

Fig. 1. Distribution of hepatitis C virus (HCV) genotypes by sex.

Mean patient age was 56.6 ± 13.6 years (± SD; range, 13–87 years; Table 1). Although no significant age differences in HCV genotypes were observed ($p > 0.05$), significant differences were observed in disease prevalence ($p < 0.001$, by chi-squared test), with the highest prevalence found in patients aged 50–59 years (26.8%; 124/463), followed by those aged 40–49 years (23.5%; 109/463) and 60–69 years (20.7%; 96/463). The lowest prevalence was detected in patients aged 10–19 years (0.6%; 3/463), and no infection was recorded in patients aged 0–9 years (Table 2).

Table 1. Distribution of the hepatitis C virus (HCV) genotypes/subtypes during the period from 2007 to 2016 in Cheonan, Korea.

HCV genotype	Sex (N, %)					
	Number of participants (N = 463)	Total (%)	Male (n)	%	Female (n)	%
1a	5	1.1	3	60	2	40
1b	209	45.1	106	50.7	103	49.3
2a	234	50.5	135	57.7	99	42.3
2b	3	0.6	1	33.3	2	66.7
3a	7	1.5	6	85.7	1	14.3
3b	1	0.2	1	100		
4a	2	0.4	1	50	1	50
6a	1	0.2	1	100		
1b + 2a	1	0.2	1	100		
Total	463		255	55.1	208	44.9

Table 2. Distribution of HCV genotypes by age.

HCV genotype	Age group (N, %)							
	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89
1a		1 (20)			3 (60.0)	1 (20)		
1b	1 (.5)	2 (1.0)	19 (9.1)	52 (24.9)	57 (27.3)	42 (20.1)	28 (13.4)	8 (3.8)
2a	2 (.9)	3 (1.3)	11 (4.7)	51 (21.8)	61 (26.1)	51 (21.8)	46 (19.7)	9 (3.8)
2b				2 (66.7)		1 (33.3)		
3a			3 (42.9)	2 (28.6)	1 (14.3)		1 (14.3)	
3b						1 (100)		
4a					2 (100.0)			
6a				1 (100)				
1b+2a (mixed)				1 (100)				
Total	3 (.6)	6 (1.3)	33 (7.1)	109 (23.5)	124 (26.8)	96 (20.7)	75 (16.2)	17 (3.7)

HCV, hepatitis C virus.

Comparison of HCV RNA level by genotype and year

The result of HCV RNA level was expressed in international units (IU)/ml, with each unit representing approximately viral load of 2.7 copies/ml according to the kit insert for RT-qPCR test used in this study. The correlations between HCV RNA level and genotype are shown in Table 3, with the most predominant genotypes being genotype 2a (mean, 2.61E+06 IU/ml, SD, 4.10E+06 IU/ml) and genotype 1b (mean, 3.15E+06 IU/ml, SD, 4.77E+06 IU/ml), with a mean difference of 0.54E+06 IU/ml. There were no significant correlations between the mean HCV RNA level and HCV genotype ($p > 0.05$; Table 3, Fig. 2).

The mean HCV RNA level was 4.18E+06 IU/ml in 2012, and it decreased significantly to 2.36E+06 IU/ml in 2014, 2.15E+0.6 IU/ml in 2015, and 2.31E+0.6 IU/ml

Table 3. HCV RNA level by genotype.

HCV genotype	Total (n = 259)	Mean	Min	Max
1b+2a (mixed)	1	1.22E+06	1.22E+06	1.22E+06
1b	104	3.15E+06	1.19E+02	3.54E+07
2a	144	2.61E+06	1.14E+03	2.24E+07
2b	3	3.56E+06	7.24E+04	1.05E+07
3a	4	7.85E+05	2.83E+05	1.16E+06
3b	1	2.02E+06	2.02E+06	2.02E+06
4a	2	1.29E+06	3.33E+05	2.24E+06

HCV, hepatitis C virus.
The unit of RNA level is IU/ml.

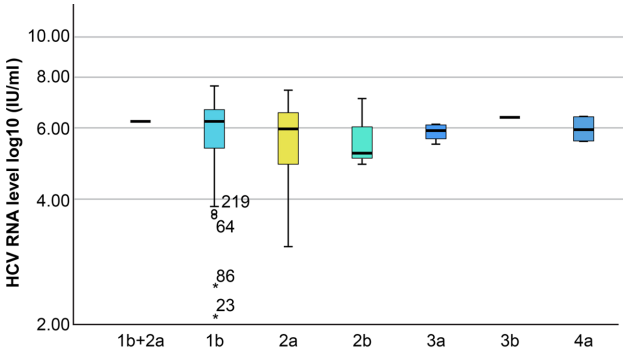

Fig. 2. HCV RNA level according to genotype is delineated. No significant correlations between the mean HCV RNA level and HCV genotype was noted.

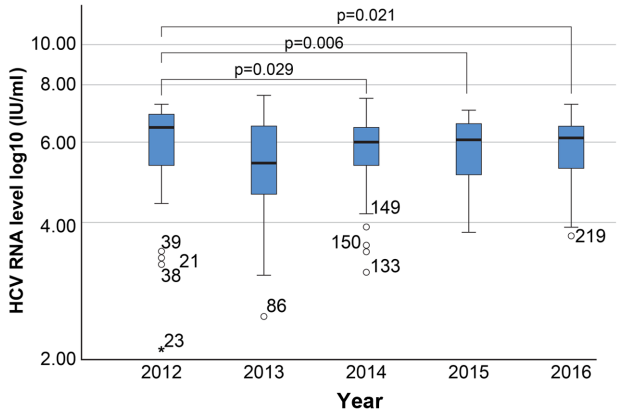

Fig. 3. HCV RNA level according to year from 2012 to 2016 is shown. Statistically significant findings between years are indicated with connected lines.

Table 4. HCV RNA level by year.

Year	Number	Mean	Min	Max
2012	46	4.18E+06	1.19E+02	1.56E+07
2013	50	3.20E+06	2.99E+02	3.54E+07
2014	58	2.36E+06	1.25E+03	2.24E+07
2015	56	2.15E+06	6.54E+03	1.10E+07
2016	49	2.31E+06	4.98E+03	1.59E+07

HCV, hepatitis C virus.
The unit of RNA level is IU/ml.

in 2016 (p = 0.029, 0.006, and 0.021, respectively; Table 4, Fig. 3).

Distribution and dynamics of HCV genotypes from 2007 to 2016

In 2007, genotype 1b was predominant (54.2%), followed by genotypes 2a (41.7%), 1a (2.1%), and 3a (2.1%). In 2016, the predominant genotypes had shifted to 2a (49.0%), 1b (46.9%), 3b (2%), and 4a (2%). Notably, subtype 1b decreased from 54.2% in 2007 and 56.4% in 2008 to 36.6% in 2009, increased significantly to 61.0% in 2010, decreased to 32.1% in 2015, and increased again to 46.9% in 2016 (p = 0.79; Fig. 4). Alternatively, subtype 2a increased from 41.7% in 2007 and 43.6% in 2008, to 56.1% in 2009, decreased to 36.6% in 2010, increased to 42.9% and 41.3% in 2011 and 2012, respectively, before increasing significantly to 60.0%, 62.1%, and 62.5% in 2013, 2014, and 2015, respectively, and then decreasing to 49% in 2016, revealing overall statistically significant differences in genotype prevalence by year (p = 0.005; Fig. 4).

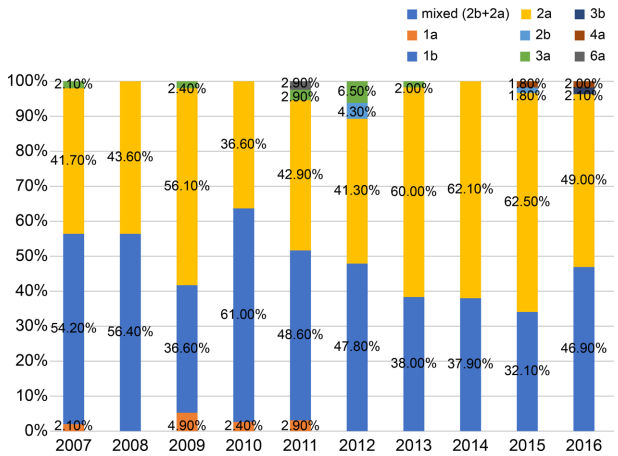

Fig. 4. Dynamics and distribution of HCV genotypes from 2007 to 2016.

Discussion

In this study, the 10-year dynamics of HCV infection, and the distribution of HCV genotypes in Cheonan, Korea were investigated. Five genotypes (1, 2, 3, 4, and 6) were detected, with genotypes 2a and 1b being the most common. The prevalence of genotype 2a increased slightly from 2007 to 2016; whereas that of genotype 1b decreased over the same period. Additionally, between 2013 and 2015, HCV genotype 2a showed a marked increase in Cheonan area. Furthermore, genotype 1b showed an increase, particularly in 2010, compared with the findings of other studies in Korea [2, 13, 14]. Thus, it was concluded that these genotypes were endemic during these years. Notably, the HCV RNA levels were not significantly correlated with genotype; however, the RNA levels of genotype 1b were higher than those of genotype 2a, and the mean HCV RNA level decreased over time.

The findings of this study are consistent with those of previous studies in Korea, insofar as the most common genotypes were genotypes 1b and 2a [2, 13, 14, 18]. Genotype 1b is globally well-distributed, being found in East Asia, Southeast Asia, America, Europe, and Canada [19–23]. Moreover, genotype 1b is predominantly distributed in East Asian countries, including Mongolia (98%), Japan (64.3%, 62.3%, 70.8%), mainland China (52.18%), and Taiwan (45.5%) [24–27]; however, a notable decrease in the prevalence of HCV genotype 1b has been observed in Japan [26]. Similarly, the present study, a decrease in subtype 1b was observed over time, as subtype 2a became predominant.

Similar to the findings here, Lee *et al.* (2008) found that HCV genotype 2a was predominant in Jeju Island, Korea [18]. HCV genotype 2 is most commonly found in West Africa and parts of South America [8, 28]. The present study showed that genotype 2a is predominant in Cheonan, similar to that in some other East Asian countries, including Japan and mainland China. Previous studies have found that HCV genotype 2 is predominant in northeast China, but its prevalence decreased from northern to southern China. Furthermore, in Japan, genotypes 2a and 2b showed prevalence rates of

17.6% and 10.3%, respectively [25, 26]. Consistent with these findings, HCV genotypes 1a, 2b, 3a, 4a, and 6a were identified in some cases in the present analysis of population in Cheonan, Korea.

In a previous Korean study, HCV genotype 3a showed a prevalence of 1%, similar to the results of the present study (1.5%) [2]. HCV genotype 3 is the next most common genotype globally, and is more frequently distributed in South Asia [8]. For example, in Malaysia, HCV genotype 3 was detected in 61.9% of cases [29]; however, both Kim *et al.* (2013) and Han *et al.* (1997) mentioned that no previous studies have reported the distribution of HCV genotype 6 in Korea [2, 13]. Seong *et al.* (2013) found that HCV genotype 6c was detected in 1% of young Korean males with chronic HCV infection, and a history of intravenous drug use and tattooing [14]. In the present study, genotype 6a was identified in a single patient (0.2%). Comparatively, HCV genotype 6 has been detected more frequently in Southeast Asian countries, such as Vietnam, Cambodia, Thailand, Myanmar, Singapore, Laos, and mainland China [21, 23, 25, 29–31]. Additionally, HCV genotypes 3 and 6 are widely distributed among intravenous drug users [32]. HCV genotype 1a is notably rare in Korea, and was detected in just five cases (1.1%) in this study. Indeed, genotype 1a is rarely found in Asia [20], however, sequence analysis confirmed that the HCV outbreak in the Korean hospital from November 2015, one of the largest healthcare-associated outbreaks ever detected in Korea (triggered through the misuse of single-use medical devices) was caused by HCV genotype 1a [33].

The present study revealed that HCV genotypes 1b and 2a markedly increased in 2010, and between 2013 and 2015, respectively, thereby showing endemic features. Previous research revealed that HCV RNA levels in patients with HCV genotype 1b were significantly higher than those in patients with HCV genotype 2a [13]. Similarly, in the present study, HCV RNA levels of genotype 1b were higher than those of genotype 2a; however, this difference was not statistically significant. Despite this result, these findings may be clinically important for improving the understanding of HCV infections.

In this study, HCV nonstructural 5A (NS5A) L31/Y93 mutation analysis using nested RT-PCR and sequencing was investigated on 57 patients with genotype 1b among enrolled 463 patients in 2015–2016 (data not shown). NS5A-Y93H were detected in 8 cases, and NS5A-L31M was found in 1 case. In 9 positive cases, 2 cases with Y93H variant had history of previous treatment failure with interferon-based regimen, and one of them had liver cirrhosis. The rest of 7 positive cases were treatment-naive patients, and no one had cirrhosis. The mean HCV RNA levels showed no significant difference between Y93H variant group and Y93 wild-type strain group ($p > 0.05$, data not shown). Resistance to HCV DAAs is an important consideration for selecting treatment regimen. NS5A-L31 and NS5A-Y93 were frequently detected resistance-associated substances (RASs) in both genotype-specific and pangenotypic DAAs failure cases [34, 35]. Although pangenotypic DAAs may retain activity against RASs at key positions including 31 and 93 in the NS5A region and showed improved therapeutic efficacy in most cases, potential RASs other than L31/Y93 were reported in treatment-naive patients as well as in patients treated with these drugs [35, 36]. The assessment of RASs in untreated HCV patients may be important for optimal drug use and monitoring treatment effectiveness, even in the era of pangenotypic DAAs.

The present study is important to help clarify the prevalence of genotypes in Korea, in addition to demonstrating the practical and clinical implications of the information. However, this study has some notable limitations. First, only the distribution of HCV genotypes in Cheonan Province, Korea was analyzed. Second, the source or route of infection was not investigated.

In summary, the prevalence of HCV genotype 1b tended to decrease over the decade from 2007 to 2016. Notable outbreaks of HCV genotype 1b occurred in 2010, and those of genotype 2a occurred from 2013 to 2015. The HCV RNA levels have been decreasing in Cheonan since 2012, and they were higher in patients with genotype 1b than in those with genotype 2a. Further studies are needed to determine the distribution and dynamics of HCV genotypes across various regions of Korea. Studies on the epidemiology of HCV, particularly the relationships between genotype and RNA levels, may also improve our understanding of optimal treatment strategies for HCV infection in this province within Korea.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. World Health Organization. 2017. Global Hepatitis Report 2017. Geneva: World Health Organization, 2017.
2. Kim DY, Kim IH, Jeong SH, Cho YK, Lee JH, Jin YJ, *et al.* 2013. A nationwide seroepidemiology of hepatitis C virus infection in South Korea. *Liver Int.* **33**: 586-594.
3. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **244**: 359-362.
4. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. 2005. *Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses*. Academic Press, Cambridge, USA.
5. Borgia SM, Hedskog C, Parhy B, Hyland RH, Stamm LM, Brainard DM, *et al.* 2018. Identification of a novel hepatitis C virus genotype from Punjab, India: expanding classification of hepatitis C virus into 8 genotypes. *J. Infect. Dis.* **218**: 1722-1729.
6. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, *et al.* 2014. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology* **59**: 318-327.
7. Simmonds P. 1997. Clinical relevance of hepatitis C virus genotypes. *Gut* **40**: 291.
8. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, *et al.* 2015. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* **61**: 77-87.
9. Kim BH, Park J-W. 2018. Epidemiology of liver cancer in South Korea. *Clin. Mol. Hepatol.* **24**: 1-9.
10. Petruzziello A, Marigliano S, Loquercio G, Coppola N, Piccirillo M, Leongito M, *et al.* 2017. Hepatitis C Virus (HCV) genotypes distribution among hepatocellular carcinoma patients in Southern Italy: a three year retrospective study. Infect. *Agents Cancer* **12**: 52.
11. Raimondi S, Bruno S, Mondelli MU, Maisonneuve P. 2009. Hepatitis C virus genotype 1b as a risk factor for hepatocellular carcinoma development: a meta-analysis. *J. Hepatol.* **50**: 1142-1154.
12. Bruno S, Crosignani A, Maisonneuve P, Rossi S, Silini E, Mondelli MU. 2007. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. *Hepatology* **46**: 1350-1356.
13. Han CJ, Lee H-S, Kim H-S, Choe JH, Kim CY. 1997. Hepatitis C virus genotypes in Korea and their relationship to clinical outcome in type C chronic liver diseases. *Korean J. Intern. Med.* **12**: 21.
14. Seong MH, Kil H, Kim JY, Lee SS, Jang ES, Kim J-W, *et al.* 2013. Clinical and epidemiological characteristics of Korean patients with hepatitis C virus genotype 6. *Clin. Mol. Hepatol.* **19**: 45-50.
15. Waheed Y, Siddiq M, Jamil Z, Najmi MH. 2018. Hepatitis elimination by 2030: progress and challenges. *World J. Gastroenterol.* **24**: 4959-4961.
16. Geddawy A, Ibrahim YF, Elbahie NM, Ibrahim MA. 2017. Direct acting anti-hepatitis C virus drugs: clinical pharmacology and future direction. *J. Transl. Int. Med.* **5**: 8-17.
17. Jeong S-H, Jang ES, Choi HY, Kim K-A, Chung W, Ki M. 2017. Current status of hepatitis C virus infection and countermeasures in South Korea. *Epidemiol. Health* **39**: e2017017.
18. Lee H, Cho YK, Kim HU, Choi EK, Hyun S, Kang D, *et al.* 2008. Distribution of hepatitis C virus genotypes in Jeju Island. *Korean J. Hepatol.* **14**: 28-35.
19. Cornberg M, Razavi HA, Alberti A, Bernasconi E, Buti M, Cooper C, *et al.* 2011. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. *Liver Int.* **31**: 30-60.
20. Greene W, Cheong M, Ng V, Yap K. 1995. Prevalence of hepatitis C virus sequence variants in South-East Asia. *J. Gen. Virol.* **76**: 211-215.
21. Li C, Yuan M, Lu L, Lu T, Xia W, Pham VH, *et al.* 2014. The genetic diversity and evolutionary history of hepatitis C virus in Vietnam. *Virology* **468**: 197-206.
22. Wasitthankasem R, Vongpunsawad S, Siripon N, Suya C, Chulothok P, Chaiear K, *et al.* 2015. Genotypic distribution of hepatitis C virus in Thailand and Southeast Asia. *PLoS One* **10**: e0126764.
23. Nouhin J, Iwamoto M, Prak S, Dousset J-P, Phon K, Heng S, *et al.* 2019. Molecular epidemiology of hepatitis C virus in Cambodia during 2016-2017. *Sci. Rep.* **9**: 7314.
24. Baatarkhuu O, Kim DY, Ahn SH, Nymadawa P, Dahgwahdorj Y, Shagdarsuren M, *et al.* 2008. Prevalence and genotype distribution of hepatitis C virus among apparently healthy individuals in Mongolia: a population-based nationwide study. *Liver Int.* **28**: 1389-1395.
25. Chen Y, Yu C, Yin X, Guo X, Wu S, Hou J. 2017. Hepatitis C virus genotypes and subtypes circulating in Mainland China. *Emerg. Microbes Infect.* **6**: e95.
26. Toyoda H, Kumada T, Takaguchi K, Shimada N, Tanaka J. 2014. Changes in hepatitis C virus genotype distribution in Japan. *Epidemiol. Infect.* **142**: 2624-2628.
27. Lee C-M, Lu S-N, Hung C-H, Tung W-C, Wang J-H, Tung H-D, *et al.* 2006. Hepatitis C virus genotypes in southern Taiwan: prevalence and clinical implications. *Trans. R Soc. Trop. Med. Hyg.* **100**: 767-774.
28. Assih M, Ouattara AK, Diarra B, Yonli AT, Compaore TR, Obiri-Yeboah D, *et al.* 2018. Genetic diversity of hepatitis viruses in West-African countries from 1996 to 2018. *World J. Hepatol.* **10**: 807-821.
29. Ho S-H, Ng K-P, Kaur H, Goh K-L. 2015. Genotype 3 is the predominant hepatitis C genotype in a multi-ethnic Asian population in Malaysia. *Hepatobiliary Pancreat. Dis. Int.* **14**: 281-286.
30. Akkarathamrongsin S, Praianantathavorn K, Hacharoen N, Theamboonlers A, Tangkijvanich P, Tanaka Y, *et al.* 2010. Geographic distribution of hepatitis C virus genotype 6 subtypes in Thailand. *J. Med. Virol.* **82**: 257-262.
31. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, Rasachak B, *et al.* 2009. Genetic history of hepatitis C virus in East Asia. *J. Virol.*

http://dx.doi.org/10.48022/mbl.2205.05003

83: 1071.
32. Chao D, Abe K, Nguyen M. 2011. Systematic review: epidemiology of hepatitis C genotype 6 and its management. *Aliment Pharmacol. Ther*. **34**: 286-296.
33. Chung Y-S, Choi J-Y, Han MG, Park KR, Park S-J, Lee H, *et al.* 2018. A large healthcare-associated outbreak of hepatitis C virus genotype 1a in a clinic in Korea. *J. Clin. Virol*. **106**: 53-57.
34. Kan T, Hashimoto S, Kawabe N, Murao M, Nakano T, Shimazaki H, *et al.* 2016. The clinical features of patients with a Y93H variant of hepatitis C virus detected by a PCR invader assay. *J. Gastroenterol*. **51**: 63-70.
35. Hong C-M, Lin Y-Y, Liu C-J, Lai Y-Y, Yeh S-H, Yang H-C, *et al*. 2021. Drug resistance profile and clinical features for hepatitis C patients experiencing DAA failure in Taiwan. *Viruses* **13**: 2294.
36. Wyles D, Luetkemeyer A. 2017. Understanding hepatitis C virus drug resistance: clinical implications for current and future regimens. *Top. Antivir. Med.* **25**: 103-109.