Original Article

Hepatitis C virus genotype diversity in Ningxia Hui autonomous region, Northwestern China

Diversidade genética do vírus da hepatite C na região autônoma de Ningxia Hui.

C. Anª [iD], W. Maª [iD], R. F. Heª [iD], T. Wuª [iD], J. N. Shiᵇ [iD], T. T. Sunᵇ [iD] and W. H. Piaoᵃ,ᵇ* [iD]

ªPeople's Hospital of Ningxia Hui Autonomous Region, Department of Laboratory Medicine, Yinchuan, Ningxia, China
ᵇNingxia Medical Uniwersity, College of Clincal Medicine, Yinchuan, Ningxia, China

Abstract

Hepatitis C virus (HCV) genotypes vary greatly in different regions. The aim of this study is to investigate the distribution of HCV genotypes in HCV infected patients, in Ningxia Hui Autonomous Region. Nucleic acid extraction and amplification were performed with test kits on 153 HCV infected patients serum samples. The HCV viral load was measured using reverse transcriptase PCR (RT-PCR) and HCV genotypes were determined. Among the 153 HCV-infected patients, 56 had genotype (GT)1b (36.60%), 45 had GT2a (29.40%), 23 had GT3a (15.00%), 14 had GT3b (9.20%),13 had GT6a (8.50%), 1 had GT1g (0.70%), 1 had GT6xa (0.70%). In GT1b, 21.40% were female and 78.60% were male; in GT2a, 42.20% were female and 57.80% were male;Males were most prevalent in genotypes 1b(39.30%), while female were most prevalent in genotype 2a(46.30%). Rare GT1g and GT6xa were also detected in males. The 41-50 year age group had the highest HCV prevalence of 32.00%. HCV GT1b is the predominant HCV genotype in Ningxia Hui Autonomous Region.

Keywords: hepatitis C, hepatitis C virus, genotype, Ningxia Hui autonomous region.

Resumo

Os genótipos do vírus da hepatite C (HCV) variam muito de acordo com a região. O objetivo deste estudo é investigar a distribuição do genótipo do vírus da hepatite C em pessoas infectadas pelo vírus na região autônoma de Ningxia Hui. A extração de ácido nucleico e a expansão de amostras séricas em 153 pacientes infectados com HCV foram realizadas utilizando kits de ensaio. A capacidade do vírus HCV foi medida pela reação em cadeia da polimerase retrotranscrição (RT-PCR) e o genótipo HCV foi determinado. Os genótipos (Gt) tiveram a seguinte distribuição entre os 153 casos de infecção HCV: 56 Gt 1-B (36, 60%), 45 Gt 2-A (29, 40%), 23 Gt 3-A (15, 00%), 14 Gt 3-B (9, 20%), 13 Gt 6-A (8, 50%), 1 Gt 1-G (0, 70%) e 1 Gt 6-Xa (0, 70%). Já o sexo dos indivíduos teve a seguinte distribuição: Gt 1-B, 21, 40% mulheres e 78, 60% homens; Gt 2-A, 42, 20% mulheres e 57, 80% homens. A presença de homens é mais comum no genótipo 1-B (39, 30%), enquanto as mulheres ocorrem mais comumente no genótipo 2-A (46, 30%). Gt 1-G e Gt 6-Xa raros também foram detectados em homens. A taxa de infecção com HCV para grupos etários de 41 a 50 anos é a mais alta, com 32,00%. HCV Gt 1-B é o genótipo dominante do HCV na região autônoma de Ningxia Hui.

Palavras-chave: hepatite C, vírus da hepatite C, genótipos, região autônoma de Ningxia Hui.

1. Introduction

The World Health Organization (WHO)'s global hepatitis strategy aims to reduce new hepatitis infections by 90% and deaths by 65% between 2016 and 2030. Hepatitis C virus (HCV) belong to the Hepacivirus genus, with six major genotypes, which can cause liver disease (Lindenbach and Rice, 2005; Bilal, et al., 2022). Determination of HCV genotypes and subtypes are important to predict response to antiviral therapy for the efficacy and barrier to resistance of non-pangenotypic direct-acting antiviral agents (DAAs) depend on the HCV genotypes and subtypes (Hayes, et al., 2022). In developing countries like China, identification of HCV genotypes and subtypes is still

lacking. The overall HCV treatment can be improved by genotyping and quantification of HCV virus, which could also be used as a good indicator for decision making of an optimal treatment regimen.

HCV genotype distribution varies in different regions (Ullah et al., 2021; Petruzziello et al., 2016; Ju et al., 2015; Sallam et al., 2019). So far, there is little literature on the distribution of HCV genotypes in Northwestern China, especially the epidemiology of different age and gender group. Herein, we invesitigated the genotype distribution of HCV-infected patients from Ningxia Hui Autonomous Region, Northwestern China, which can provide a basis

for further study of HCV prevalence in the region and also guidance for individualized treatment.

2. Materials and Methods

A total of 153 HCV RNA positive serum samples from 2019 to 2021 enrolled in the further HCV genotyping which were obtained from individuals who were diagnosed as chronic hepatitis C and conducted relevant inspections at the Department of Laboratory Medicine, People's Hospital of Ningxia Hui Autonomous Region, China. The study was approved by the Ethics Committee of the People 's Hospital of Ningxia Hui Autonomous Region. Written consent were all acquired from the patients. The baseline data including age, gender, and prior surgical history were collected from Laboratory Information System (LIS) and the Hospital Management Information System (HIS).

For high precision HCV-RNA quantitative detection (PCR-Fluorescence Probing) 153 enrolled serum samples were processed using Rotor-Gene Q/Thermo-optical Analyzer and supporting diagnostic kit. Data were automatically analyzed by Rotor-Gene Q software. All the above procedures were strictly conducted in accordance with the reagent and instrument instructions.

For HCV genotype and subtype analysis, the HCV RNA was extracted by relevant HCV genotyping kit (PCR-fluorescence probe method), Da AN Gene Biotechnology Co, Ltd, Shenzhen, China. The HCV Genotyping Kit, with sensitivity of $5.0×10^3$ IU/mL, a region within the HCV-RNA 5′UTR was amplified and identified the five most common HCV genotypes (1b, 2a, 3a, 3b, 6a). The extracted target genes were amplified by T100 Thermal Cycler (Bio-Rad, Hercules, California, USA). Amplification was performed according to the following program conditions: 50°C for 25 minutes, 95°C for 15 minutes, 94°C for 30 seconds, 55°C for 40 seconds, 72°C for 45 seconds, 45 cycles. After the reverse transcription-PCR completed,HCV genotypes and subtype were determined in accordance with the relevant instructions. In addition, the unascertained HCV genotypes and subtypes were sequenced using the Diagnostic Kit For HCV Genotyping (PCR-sequencing Analysis) (Life-DaAn Diagnostic Products Technology Co., Ltd.) and Applied Biosystems 3730 gene sequencer. The sequencing results were analyzed using Chromas software 2.6.6 (Technolysium Ltd.).

The distribution of the HCV genotype and the average viral load were calculated using Microsoft Excel. No additional statistical tests were performed, due to the small sample size.

3. Results

In this study, four HCV genotypes (1, 2, 3 and 6) and six subtypes (1b, 2a, 3a, 3b, 6a, 6xa and 1g) were found in 153 patients with chronic HCV infection, and no mixed genotype infection was found. The most common HCV subtype was 1b (36.60%), followed by 2a (29.41%), 3a (15.03%), 3b (9.15%), and 6a (8.51%). In addition, we

found rare subtypes 1g (0.65%) and 6xa (0.65%) by PCR and sequencing (Figure 1).

HCV 1b was the most prevelant genotype in Ningxia Hui Autonomous Region and all of the other areas in China (Table 1).

We found gender variance for HCV genotypes. In GT1b, 21.40% (12/56) were female and 78.60% (44/56) were male; in GT2a, 42.20% (19/45) were female and 57.80% (26/45) were male; in GT3, only 18.90% (7/37) were female and 81.10% (30/37) were male; and in GT6a, 23.10% (3/13) were female and 76.90% (10/13) were male. Males (39.30%, 44/112) were most prevalent in genotypes 1b, while females (46.30%,19/41) were most prevalent in genotype 2a. Genotype 1g and 6xa were also found in males. In addition, we also found that the proportion of males was higher than that of females in all seven HCV gene subtypes (Figure 2).

The highest number of HCV infection was between the age of 41–50 years (32.00%). HCV genotype 1b, 3a, 3b and 6a was most common in 41-50 age group (40.60%);
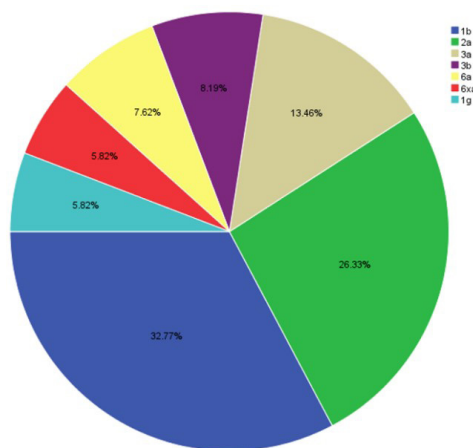

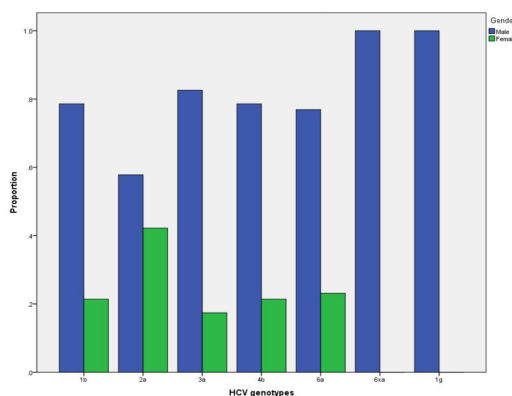**Figure 1.** Distribution of HCV genotypes in Northwestern China.

Figure 2. Proportion of HCV genotypes by gender in Northwestern China.

Table 1. HCV genotype distribution in different regions of China.

Region	HCV genotype and subtype(%)								Reference
	1b	2a	3a	3b	6a	6n	others	Total(n)	
Northeastern	64	33	0	1	1	0	1	111	Ju et al. (2015)
Western	63	21	3	3	0	1	9	124	Ju et al. (2015)
Southern	61	15	7	2	15	0	0	59	Ju et al. (2015)
Centra	78	20	0	0	0	0	2	40	Ju et al. (2015)
Eastern	77	8	4	2	3	1	4	275	Ju et al. (2015)
Northern	67	22	3	2	1	0	5	403	Ju et al. (2015)
Sichuan province	76	7	3	9	5	0	0	469	Hu et al.(2021)
Northwestern (this study)	37	29	15	9	8	0	1	153	

Table 2. HCV genotypes and viral load in different age groups.

|	HCV genotype and subtype						
	1b	2a	3a	3b	6a	6xa	1g
Age (years)							
21-30	3	2	1	0	1	0	0
31-40	13	3	6	1	3	0	0
41-50	17	5	9	11	6	1	0
51-60	15	12	7	2	2	0	1
61-70	6	12	0	0	0	0	0
71-80	2	9	0	0	1	0	0
81-90	0	2	0	0	0	0	0
Average viral load (IU/ml)	4.14E+06	5.35E+06	12.25E+06	1.98E+06	7.17E+06	5.53E+06	4.54E+06

type 2a was most common in the 51-70 age group (53.30%). The average viral load of HCV 3a (12.25E+06 IU/ml) was also higher than that of other six HCV gene subtypes (Table 2).

We also found that the constituent ratio of HCV genotypes (1b, 2a, 3a, 3b, 6a, 6xa, 1g) in Han patients with HCV infection was higher than that in Hui patients(Figure 3).

4. Discussion

HCV infection causes a major threat to the global public health (Mohd et al., 2013). It is estimated that there were 10 million HCV-infected people in China in 2019. The distribution of HCV genotype and subtype have obvious geographical and population differences around the world. Different HCV genotypes and subtypes respond differently to specific treatment regimens.It is important to clarify the prevalenct HCV genotype in Ningxia Hui Autonomous Region, Northwestern China since few data are currently available concerning the distribution of HCV in Northwestern China.

The changes of HCV genotype/subtype and viral load have important guidance value for optimal clinical treatment. Experts recommend that HCV infected persons
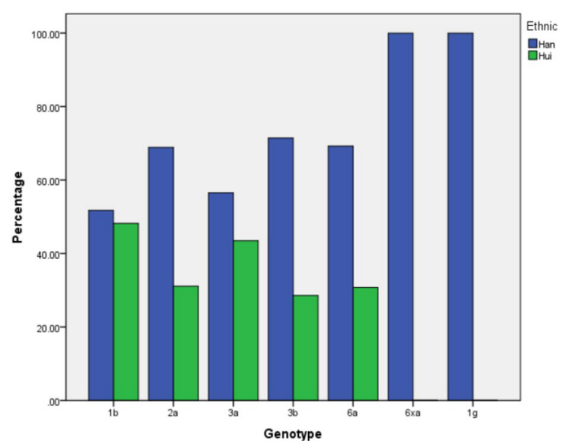
Figure 3. Distribution of HCV genotypes in Han and Hui ethnic groups patients with hepatitis C in Northwestern China.

should be genotyped before starting antiviral treatment, mainly considering that its affordability in China is better than pan-genotyping scheme. Previously, In China, HCV 1b

(56.80%) was the predominant HCV genotypes, followed by HCV genotype 2, 3, 6. Additionally, HCV genotype 4 and 5 were rare among the whole of China. While in southern and western China, genotype 1b was the main type, and genotype 2, 3 and 6 also accounted for a large proportion. In genotype 3, the prevalence of genotype 3b was higher than that of 3a. Genotype 3 and 6 HCV infection exists nationwide and no longer restricted to southern and western China (Ju et al., 2015). It is worth noting that the epidemic pattern of HCV in China is evolving due to the change of population mobility.

Our study found that in northwestern China the most common subtype was 1b (36.60%), followed by 2a (29.41%), 3a (15.03%), 3b (9.15%), and 6a (8.51%). That is slightly different from other areas in China. The proportion of HCV genotype 1b (36.60%) in Ningxia Hui Autonomous Region was lower than that in Sichuan Area(76.10%) and Liaoning area(45.10%) (Li et al., 2021; Hu et al., 2021), but the proportion of 3a (15.03%) was higher than these areas. In addition, we found HCV genotype 1g (0.65%) and 6xa (0.65%) by PCR and sequencing, which were rare in Northwestern China. Ningxia Hui Autonomous Region is an underdeveloped inland region in northwestern China. Diversity of HCV gene subtypes in the region brings new challenges for the prevention and control of HCV and other various infectious diseases. In addition, there were few studies on HCV genotype distribution in northwestern China. Therefore, our study will play a key role in the diagnosis, treatment and prognosis of HCV in Ningxia Hui Autonomous Region.

In every group of our study, the incidence in males was higher than in females, and 95.40% of all positive cases were aged over 30 years, mostly between 41-50 years of age (32.00%). The economic development in Ningxia Hui Autonomous Region was unbalanced, people knew less about hepatitis C. The onset of hepatitis C is insidious and chronic, and about 20.00% of patients with hepatitis C can selfheal, mainly because these patients have strong autoimmune function and the amount of infected virus is relatively small.

The viral load of HCV is related to its genotype, and the viral load are of great value for guiding clinical treatment. The mean viral load among patients with HCV genotype 1 infected was reported to be higher than that of infected with genotype 4 (Mishra et al., 2020). However, our research shows that the HCV RNA level of HCV-3a patients is higher than that of other genotypes (Table 2). Previously, Brady and Stoykova (2019) reported that the mean viral load level with HCV genotype 3 is higher than that of others (Brady and Stoykova, 2019). We think that these different research results may be caused by the heterogeneity of the populations studied.

Our research shows that in northwestern China, Ningxia Hui Autonomous Region, the constituent ratio of all the detcted HCV genotypes (1b, 2a, 3a, 3b, 6a, 6xa, 1g) in Han chronic hepatitis C patients were higher than that in Hui patients. We thought that of the Chinese population, 64.05% are of Han nationality and 35.04% are of Hui nationality in Ningxia Hui Autonomous Region, which accounts for the results as mentioned above. Additionally, we found that HCV genotypes in the Hui chronic hepatitis

C patients were diverse. In contrary, Li et al.' s reported that the genotype of hepatitis C in Liaoning Province, the Hui nationality,only 3 genotypes were prevalent for Hui patients: GT1b, GT2, and GT3a (Li et al.,2021). Our results were slightly inconsistent with the study of Li et al for there were few amounts Hui chronic hepatitis C patients in Liaoning province. In addition, it is also possible that the results were slightly different due to geographical differences. Liaoning is located in the northeast of China, while Ningxia is located in the northwest of China.

5. Conclusion

This research reveals the current HCV genotype distribution in Ningxia Hui Autonomous Region, Northwestern China. The results provide a basis for further studying the epidemic situation of hepatitis C virus in Ningxia Hui Autonomous Region and developing targeted prevention and control strategies. It is necessary to conduct extensive research on the distribution of HCV genotypes in Ningxia Hui Autonomous Region for against HCV infection in Ningxia Hui Autonomous Region.

Acknowledgements

References

BILAL, M., BASHIR, H., AMEEN, R., SUMRIN, A., HUSSAIN, M. and MANZOOR, S., 2022. Anti HCV activity and expression inhibition of HCC markers by protein extract from Iberis gibraltarica. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, pp. e252676. http://dx.doi.org/10.1590/1519-6984.252676. PMid:35384980.

BRADY, Z. and STOYKOVA, Z., 2019. Hepatitis C virus genotype analysis in patients with chronic hepatitis in North Eastern Bulgaria. *Journal of Drug Assessment*, vol. 8, no. 1, pp. 146-149. http://dx.doi.org/10.1080/21556660.2019.1654484. PMid:31552145.

HAYES, C.N., IMAMURA, M., TANAKA, J. and CHAYAMA, K., 2022. Road to elimination of HCV: clinical challenges in HCV management. *Liver International*, vol. 42, no. 9, pp. 1935-1944. http://dx.doi.org/10.1111/liv.15150. PMid:34967486.

HU, H.H., ZHANG, C., XIE, C.B., LIU, S.H., REN, Y., DU, Y.F., YIN, L.C., DU, L. and JIANG, W., 2021. Prevalence and genotype distribution of hepatitis C virus from 1,668 individuals of Sichuan Area in China. *Clinical Laboratory*, vol. 67, no. 1, pp. 42-47. http://dx.doi.org/10.7754/Clin.Lab.2020.200335. PMid:33491428.

JU, W., YANG, S., FENG, S., WANG, Q., LIU, S., XING, H., XIE, W., ZHU, L. and CHENG, J., 2015. Hepatitis C virus genotype and subtype distribution in Chinese chronic hepatitis C patients: nationwide spread of HCV genotypes 3 and 6. *Virology Journal*,

vol. 12, no. 1, pp. 109. http://dx.doi.org/10.1186/s12985-015-0341-1. PMid:26206422.

LINDENBACH, B.D. and RICE, C.M., 2005. Unravelling hepatitis C virus replication from genome to function. *Nature*, vol. 436, no. 7053, pp. 933-938. http://dx.doi.org/10.1038/nature04077. PMid:16107832.

LI, R., XIE, Y., LIU, W. and MA, Y., 2021. Hepatitis C virus genotype and subtype distribution among ethnic minorities in Liaoning Province of China. *Medicine*, vol. 100, no. 2, pp. e24137. http://dx.doi.org/10.1097/MD.0000000000024137. PMid:33466187.

MISHRA, B.K., YADAV, U.N., KHATIWADA, S., TAMANG, M.K., DAHAL, S. and LI, Y.P., 2020. Hepatitis C virus genotype and its correlation with viral load in patients from Kathmandu, Nepal. *Journal of Infection in Developing Countries*, vol. 14, no. 12, pp. 1470-1474. http://dx.doi.org/10.3855/jidc.10391. PMid:33378293.

MOHD, H.K., GROEGER, J., FLAXMAN, A.D. and WIERSMA, S.T., 2013. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology*, vol. 57, no. 4, pp. 1333-1342. http://dx.doi.org/10.1002/hep.26141. PMid:23172780.

PETRUZZIELLO, A., MARIGLIANO, S., LOQUERCIO, G., COZZOLINO, A. and CACCIAPUOTI, C., 2016. Global epidemiology of hepatitis C virus infection: an up-date of the distribution and circulation of hepatitis C virus genotypes. *World Journal of Gastroenterology*, vol. 22, no. 34, pp. 7824-7840. http://dx.doi.org/10.3748/wjg.v22.i34.7824. PMid:27678366.

SALLAM, M., BATARSEH, R., NATSHEH, A., ABBADI, J., AL-FRAIHT, E., YASEEN, A., KADDOMI, D., KHAMEES, N., MAHAFZAH, A. and SAHIN, G.O., 2019. An update on hepatitis C virus genotype distribution in Jordan: a 12-year retrospective study from a tertiary care teaching hospital in Amman. *BMC Infectious Diseases*, vol. 20, no. 1, pp. 3. http://dx.doi.org/10.1186/s12879-019-4735-3. PMid:31892307.

ULLAH, N., KAKAKHEL, M.A., BAI, Y., XI, L., KHAN, I., KALRA, B.S., KUMAR, T., AHMAD, H., SHAH, M., GUANLAN, L. and ZHANG, C., 2021. Prevalence of active HCV infection and genotypic distribution among the general population of district Mardan, Pakistan. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e244977. http://dx.doi.org/10.1590/1519-6984.244977. PMid:34287506.