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ORIGINAL ARTICLE

Prevalence and Genotype Distribution of Hepatitis C Virus from 1,668 Individuals of Sichuan Area in China

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SUMMARY

Background: **Hepatitis C virus (HCV) is one of the main causes of liver fibrosis, chronic hepatitis, and liver cirrhosis. The aim of this study is to determine the prevalence of HCV, age-dependent prevalence and genotypes distribution in a large number of clinical samples in Sichuan area of China.**
Methods: **In the past five years from 2014 to 2018, a total number of 4,508 individuals received the serum HCV-RNA analysis in the Sichuan Provincial People's Hospital. Viral nucleic acid was extracted from the serum samples and amplified using COBAS AmpliPre/COBAS TaqMan Detection Platform. Five HCV genotypes (1b, 2a, 3a, 3b, and 6a) of serum samples from 469 HCV positive individuals collected from 2016 to 2018 were analyzed using the PCR-fluorescence probe technique.**
Results: **A total of 1,668 individuals had positive results by high precision HCV-RNA quantitative technique, corresponding to a crude prevalence of 37.0% (95% confidence interval: 33.6 - 40.3%). The majority of HCV positive individuals were aged over 41 years, accounting for 80.7% (1,346/1,668, CI: 72.3 - 87.1%). Among the nine age groups, the 41 - 50-year age group had the highest HCV prevalence of 29.8% (497/1,668, CI: 25.6 - 32.3%). Of the 469 HCV-RNA positive serum samples collected in 2016 - 2018, genotype 1b was the most frequent type found in 357 individuals, corresponding to a prevalence of 76.1% (CI: 72.3 - 80.0%).**
Conclusions: **Positive rates of HCV in the years of 2014 to 2018 showed a downward trend year by year, of which a majority of positive cases were aged over 41 years. HCV was distributed with multi-genotype features while genotype 1b yielded a very high prevalence in the Sichuan area. The results have potential for prevention and treatment of HCV infection, as well as epidemiological research.**
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KEY WORDS

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INTRODUCTION

Hepatitis C virus (HCV) is one of the main pathogenic factors of liver fibrosis, chronic hepatitis, liver cirrhosis, and other diseases, and the incidence is increasing yearly [1]. A previous study showed that the prevalence of HCV depended on countries or regions, with HCV

cases representing lower than 2% of the national population in developed countries and higher than 15% in developing countries [2]. A recent research from WHO showed that an estimated 8.9 million people, or 0.6% of the overall population in China were infected with HCV, and deaths caused by HCV-infection accounted for more than half of the world's annual liver cancer fatalities [3]. Thus, effective diagnosis and treatment of hepatitis C is seen to be a very important way to avoid disease progression and reduce the risk of multiple extrahepatic complications.

In recent years, along with the progress in technology of HCV molecular detection and the availability of more effective, curative direct-acting antiviral medications, hepatitis C elimination has become an imaginable goal for the first time [4]. Clinically, HCV-RNA detection should be carried out promptly for an individual who is anti-HCV positive, and a test of the dominant genotype lays a solid foundation to guide clinical treatment. In addition, HCV genotyping plays a very important role in investigation of infection outbreaks and improvement of the general understanding of the virological characteristics of hepatitis C. PCR-fluorescence probe typing, which is widely used in clinical laboratories, has a high accuracy in the identification of common HCV genotypes [3].

This study explored the basic features of 1,668 individuals whose results of high precision HCV-RNA quantitative detection were positive in the Sichuan Provincial People's Hospital from 2014 to 2018. Genotype distribution of HCV during the period from 2016 to 2018 was also analyzed. The data which have a great significance in the treatment of hepatitis C provide an adequate resource to assess the prevalence of HCV and the genotype distribution in the Sichuan area.

MATERIALS AND METHODS

Serum samples from 2014 to 2018 were obtained from individuals who conducted relevant inspections at the Department of Laboratory Medicine, Sichuan Provincial People's Hospital, China. Ethical approval for the study was obtained from the Sichuan Provincial People's Hospital. Informed consent was issued by each patient before the samples and survey information was also collected. A total of 4,508 individuals received a high precision HCV-RNA detection. Among them, 469 cases between 2016 and 2018 were found to be HCV positive and were enrolled in the further HCV genotyping. All methods were conducted in accordance with relevant guidelines and regulations.

When carrying out the high precision HCV-RNA quantitative detection, serum samples were preprocessed by the COBAS AmpliPre/COBAS TaqMan Detection Platform (Roche Molecular Systems, Pleasanton, CA, USA) and supporting reagent kit of COBAS AmpliPrep/ COBAS TaqMan HCV Quantitative Test (Roche Molecular Systems). The HCV-RNA was extracted and amplified using a COBAS TaqMan nucleic acid analyzer (Roche Molecular Systems). Data were automatically collected and analyzed by Amplilink software (Roche Molecular Systems).

For HCV genotype analysis, the serum viral RNA was extracted using a HCV genotyping kit (PCR-fluorescence probe method) which was produced by Taipu Bioscience Co., Ltd, Xiamen, China. Target genes were amplified by ABI 7500 fluorescent PCR instrument (Life Technologies Holdings Pte. Ltd., Singapore). In brief, 10 μL RNA extraction product or quality control was added into the 40 μL reaction system that included primers (genotype 1b, genotypes 2a/6a and genotypes 3a/3b), HCV RT-PCR reaction solution and enzyme system, respectively. Amplification was carried out according to the following program conditions: 42°C for 30 minutes, 95°C for 3 minutes, 94°C for 20 seconds, 55°C for 20 seconds, 72°C for 30 seconds, 10 cycles; 15 seconds at 94°C, 45 seconds at 60°C, 30 cycles. Parameters of fluorescence probe were set as: all detection wells were set to FAM-TAMAR and JOE-TAMAR, and the fluorescence signal was collected at 60°C for 45 seconds. After the reaction was completed, HCV genotypes were determined in accordance with the manufacturers' instructions.

RESULTS

Among the 4,508 individuals who received a high precision HCV-RNA test, 1,668 were found to be HCV positive, giving a crude prevalence of 37.0% (95% confidence interval: 33.6 - 40.3%), 838 were females accounting for 50.2% (838/1,668), and 830 were males accounting for 49.8% (830/1,668). The percentage of HCV positive individuals in 2014, 2015, 2016, 2017, and 2018 were 52.9% (45/85, CI: 45.6 - 56.9%), 48.9% (363/742, CI: 42.8 - 52.7%), 39.9% (432/1,082, CI: 35.6 - 42.4%), 32.2% (472/1,466, CI: 25.4 - 37.1%), and 31.4% (356/1,133, CI: 25.9 - 36.8%), respectively (Figure 1). The proportion of HCV positive females increased year by year, from 42.2% (19/45) in 2014 to 56.2% (200/356) in 2018 (Figure 2). The highest HCV prevalence was 29.8% (497/1,668, CI: 42.8 - 52.7%) in the 41 - 50 year age group, followed by the 51 - 60 year age group of 25.5% (426/1,668, CI: 22.3 - 27.9%), and then the 61 - 70 year age group of 14.3% (239/1,668, CI: 12.5 - 16.1%). The majority of HCV positive cases were aged over 41 years, accounting for 80.7% (1,346/ 1,668). Among the individuals aged from 21 to 40 years, the HCV positive number was 316 accounting for 18.9% (316/1,668). Six young individuals under 20 years were HCV positive accounting for only 0.4% (6/ 1,668) (Figure 3).

Five dominant HCV genotypes including 1b, 2a, 3a, 3b, and 6a were detected among the 508 HCV-RNA positive samples collected between 2016 and 2018, of which 469 samples (92.3%) were successfully genotyped, 223 (47.5%) were males and 246 (52.5%) were

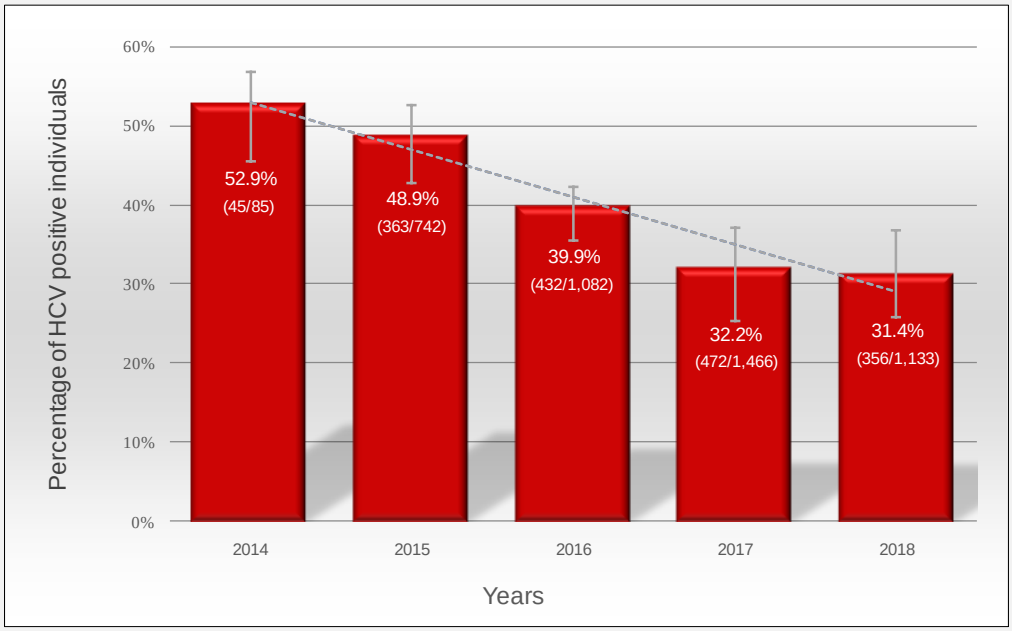

Figure 1. Proportion of HCV positive individuals from 2014 to 2018.

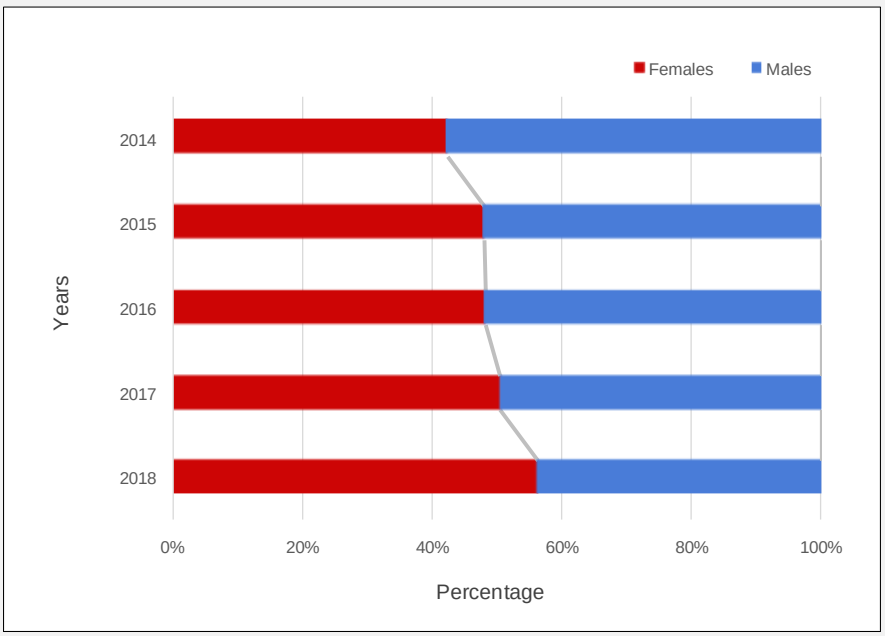

Figure 2. Difference of proportion for HCV positive cases between females and males from 2014 to 2018.

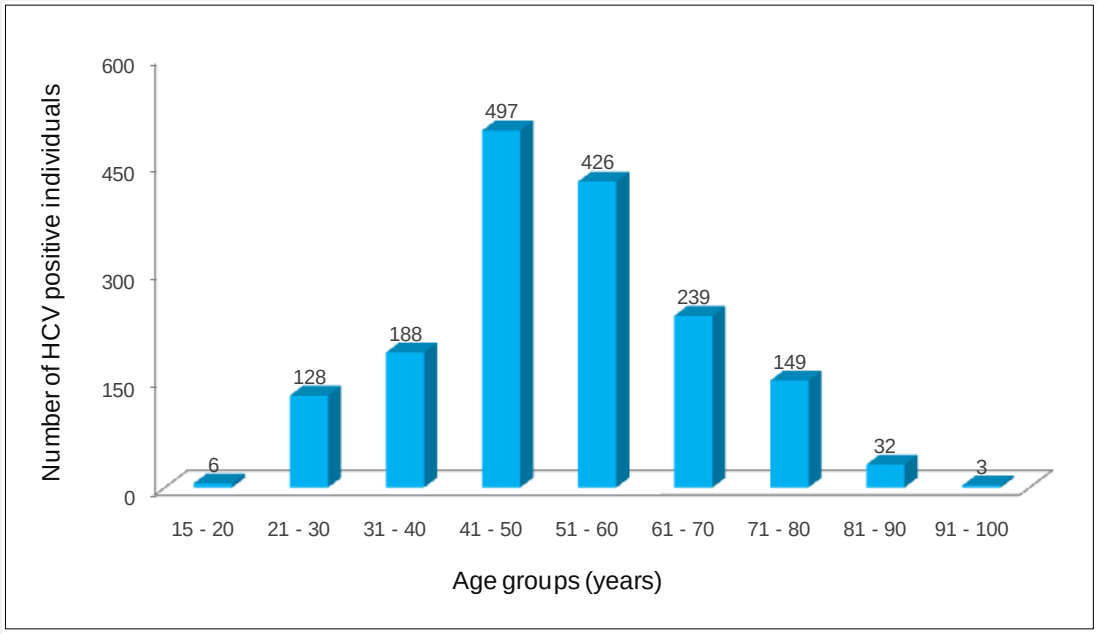

Figure 3. Age distribution of 1,668 HCV-RNA positive individuals.

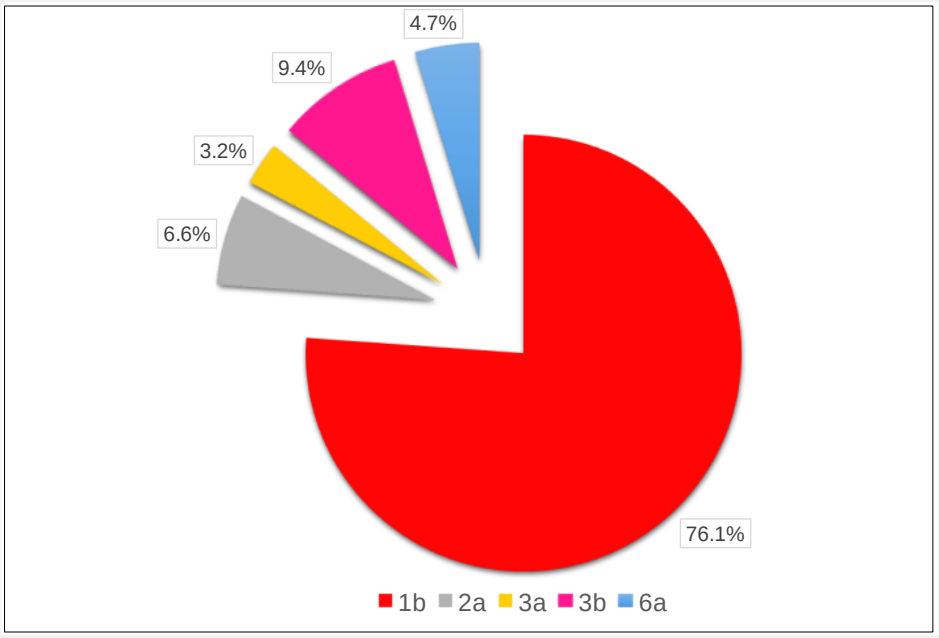

Figure 4. Distribution of five HCV genotypes from 469 HCV-positive individuals.

The most frequent genotype was 1b, followed by 3b, and then 2a, 6a, and finally 3a.

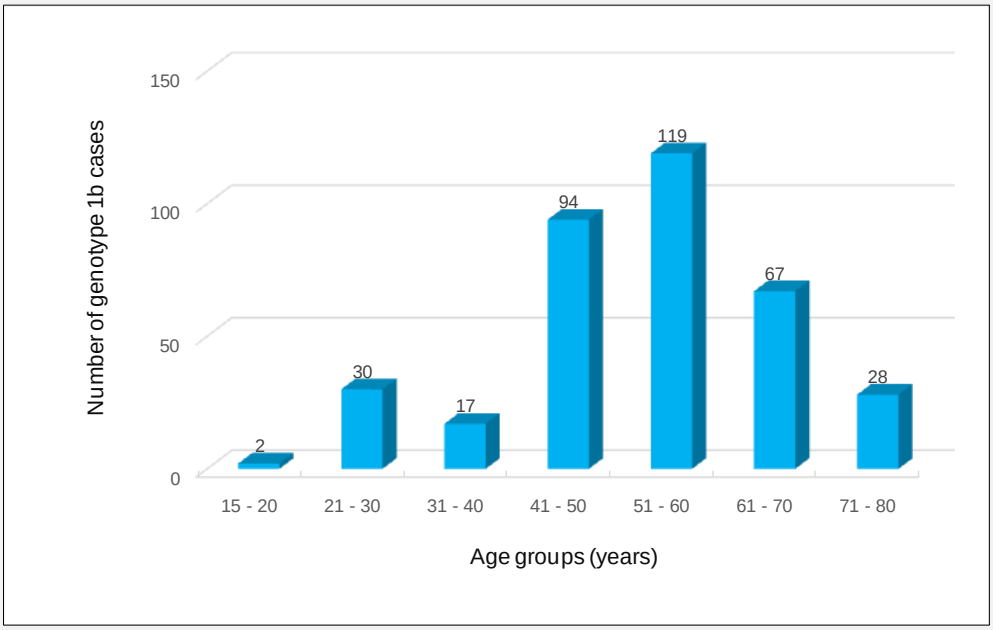

Figure 5. Genotyping results of HCV-1b by age groups from 357 HCV positive cases.

females. The most frequent genotype was 1b accounting for 76.1% (357/469), including 168 males (47.1%) and 189 females (52.9%), followed by 3b (n = 44, 9.4%), and then 2a (n = 31, 6.6%) (Figure 4). The majority of individuals infected with genotype 1b were over 41 years old, which accounted for 86.3% (308/357) (Figure 5).

DISCUSSION

It is estimated that approximately 350,000 deaths are caused by HCV infection each year, which has become a serious global public safety issue, and the viral distribution and infection rate have obvious geographical and population differences around the world [5-7]. From 2014 to 2018, a total of 4,508 individuals received the HCV-RNA quantitative detection at the Sichuan Provincial People's Hospital. Our results showed that 1,668 cases were HCV positive, accounting for 37.0% of the total number of subjects, and 80.7% of all positive cases were aged over 41 years. Although participants of HCV-RNA detection were few in the first year of 2014, the proportion of HCV positive cases between 2014 - 2018 also reflected a gradually decreasing trend to a certain extent, which indicated the measures of prevention and control for the spread of hepatitis C disease of Sichuan area in China were effective during these years. Meanwhile, the proportion of females among the HCV positive cohorts increased year by year, and correspondingly, the proportion of HCV positive males decreased yearly, which might be caused partly by the negative conversion rate of HCV in men, is higher than that in women as a former research proved [8].

Once hepatitis C is diagnosed and hepatic fibrosis occurs, anti-viral therapies should be initiated promptly. Since different HCV genotypes have diverse effects on the clinical manifestations of hepatitis C and the severity of liver disease, different treatments should be considered for the patients with different HCV genotypes [9-11]. According to the sequence homology of HCV nucleotides, this virus can be subdivided into seven major genotypes and more than 60 genetic subtypes [12]. Five HCV genotypes, which included 1b, 2a, 3a, 3b, and 6a, were analyzed from 469 individuals from 2016 to 2018 in this study. Our data showed that HCV-1b was a dominant genotype, accounting for 76.1%, which is higher than that of a prevalence of 62.8% in the study of Zhang et al. [13]. Another study regarding distribution of HCV genotypes of Putian City in south-eastern China showed the HCV-1b prevalence of 59.8%, which is much lower than that in our study [14]. Those data indicate that the prevalence of type HCV-1b in Sichuan area is much higher than in other regions.

HCV-RNA is a reliable index of viral replication, which directly reflects the existence of HCV from a genetic diagnosis perspective. This index has to be combined with anti-HCV and traditional liver function biochemical

tests (such as AST, ALT, and TBIL) in order to aid judgment. In addition, HCV genotyping methods have evolved into dozens, the most reliable of which is sequence analysis, which is the "gold standard" of HCV typing. However, this is time consuming and laborious; therefore, PCR fluorescent probe typing was used in the study. This article still lacks systematic research on HCV transmission mode and heredity in some specific populations, and HCV subtypes of the 469 samples also deserve further study.

Controlling the spread of HCV has become a very serious public health problem. In particular, the public's awareness of hepatitis C should be further improved, especially for middle aged and elderly people. It is necessary to conduct extensive and continuous research on the distribution of HCV genotypes and subtypes in Sichuan Province in the future. Results shown in this study can serve as a reference for the development of hepatitis C prevention and treatment measures, as well as public health investment plans of the Sichuan area in China.

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Ethics Approval and Consent to Participate:
Ethical approval for the study was obtained from the Sichuan Provincial People's Hospital, China. Informed consent was issued by each patient before the samples and survey information were collected.

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Declaration of Interest:
The authors declare that they have no competing interests.

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