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Analysis of Transmission Routes of Hepatitis C Virus Based on Virus Genotyping in 341 Cases with Different Suspected Initial Infection Time Points in Hunan Province, China

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Background: Few investigations have been reported on the changing trends in transmission routes of hepatitis C virus (HCV) and the corresponding HCV genotype (GT) distribution in Hunan province, China.

Material/Methods: HCV GTs, suspected viral transmission routes, and time of initial infections were investigated in 341 HCV-infected patients in 2016.


Results: Genotype 1 (GT1) (72.1%) was the most prevalent HCV GT, followed by GT6 (17.6%), GT3 (7.6%), and GT2 (2.6%). GT4 and GT5 were not found. The predominant HCV transmission routes were blood-related routes (57.5%) and intravenous drug use (IDU) (15.0%); 52.2% of the patients got HCV infection before 1994, 25.6% from 1994 to 1998, and 22.2% after 1998; 93.5% of the infections via blood-related transmission routes were with HCV GT1, 61.5% via IDU or feculent sexual contact were with HCV GT6, and 50.0% via non-healthcare invasive procedures were with HCV GT6. HCV infections via IDU or feculent sexual behavior were more prevalent in young males, while infections via invasive cosmetic procedures occurred more in young females, and both had a shorter time interval from suspected infection to confirmed clinical diagnosis. Multinomial logistic regression confirmed the time points of the initial HCV infections and suspected viral transmission routes were correlated with HCV GT distribution.

Conclusions: HCV GT1 infections via blood-related transmission routes in Hunan province have continually decreased since 1994. However, younger patients infected with HCV, especially with HCV GT6 via IDU, feculent sexual behavior, and non-healthcare invasive procedures, have significantly increased.

MeSH Keywords: **Disease Transmission, Infectious • Epidemiology • Genotype • Hepacivirus**

Abbreviations: **HCV** – hepatitis C virus; **GT** – genotype; **IDU** – intravenous drug use; **HCC** – hepatocellular carcinoma; **RNA** – ribonucleic acid; **PCR** – polymerase chain reaction; **RT-PCR** – reverse transcription-PCR; **SD** – standard deviation; **OR** – odds ratio; **CI** – confidence interval; **ANOVA** – analysis of variance; **TSICD** – time interval from suspected infection to confirmed clinical diagnosis made on visiting hospitals for HCV infection; **IFN** – interferon; **DAA** – direct-acting antiviral agent

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Background

Hepatitis C is a global pandemic disease and is moderately prevalent in China [1–6]. Most acute hepatitis C virus (HCV) infections are not given timely antiviral treatment, eventually develop into chronic infections, and subsequently result in persistent hepatic inflammation and progressive liver fibrosis, even life-threatening cirrhosis and hepatocellular carcinoma (HCC).

HCV strains prevailing in different regions are widely divergent [3–4,6–9]. Prevailing patterns of HCV are evolving in China due to changes in transmission modes and other factors like immigration and global travel [10,11]. For example, HCV genotype 2 (HCV GT2), the second predominant HCV strain following HCV GT1 in north and central China, is prevailing in southern and western provinces of China at a relatively lower level, while HCV GT6 is increasingly prevalent in south China, and HCV GT3 is thriving in southwest China [6–8,12–14]. HCV GT6 has recently migrated from Guangdong province to other provinces in the southern half of China [12].

Epidemiological studies on the regional prevalence characteristics of HCV transmission and genotypes are conducive to developing reasonable prevention and control measures of hepatitis C [6,9]. Genotyping of the virus before anti-HCV treatment is essential for personalized adjustment of therapeutic regimen [5,6,15,16].

Although patients from Hunan province have previously been recruited in several large nationwide surveys on HCV infection in China, the number of Hunan participants was limited and the data mainly focused on cross-sectional surveys on HCV GT distribution [10,11]. Few investigations have reported on suspected HCV transmission routes based on virus genotyping in different periods in Hunan province. Here, we document an investigation in 341 patients with confirmed diagnosis of hepatitis C, from which we could discern the changing trend of HCV transmission routes and its genotypes in Hunan province in recent decades.

Material and Methods

Study participants

The suspected transmission routes of HCV were surveyed by questionnaires in all the patients who visited the Second Xiangya Hospital, Central South University in Hunan province in 2016 for HCV genotype detection.

For the individualized questionnaire survey, only the investigator and the respondent were present. The rights and interests of the respondents were fully protected according to the

Ethics Committee-approved proposal. The name, gender, age, and identity card address of the respondents were confirmed and recorded for statistical analysis. In order to fully reveal the local HCV infection status, ethnicities and occupations of the patients, as well as their serum liver function indexes and complications such as liver cirrhosis, HCC, and other diseases, were not considered in the exclusion criteria.

The questionnaire consisted of the following information: contact information such as telephone numbers, where and when the suspected HCV infection occurred, the suspected transmission routes of the virus, and the time of the first confirmed HCV infection diagnosis. The respondents could refuse to answer any of the above questions.

Finally, 341 patients (180 males and 161 females) with census registry in Hunan province were enrolled in data analysis. The 341 patients came from all of the 14 districts of Hunan province and were confirmed to be infected with HCV when they were in the province. There were 327 who could recall when they were diagnosed as being HCV-infected for the first time; 301 patients responded to the suspected transmission routes of HCV while 24 gave 'unknown' answers; 230 of the 301 patients also recalled the time of the suspected HCV infection; 222 of the 230 patients recalled the time of the first confirmed diagnosis of hepatitis C infection for calculating the approximate latent period; and 16 patients were reluctant to answer the question about suspected transmission routes of HCV.

Collection of all the demographic data, clinical features, and biological samples, including the peripheral blood of the research objects, fulfilled the requirements of medical ethics. The Ethics Review Committee of the Second Xiangya Hospital of Central South University approved the study. Guidelines based on the Declaration of Helsinki set by the committee were strictly followed. All the participants provided informed written consent prior to study enrollment.

Diagnosis of HCV infection and genotyping of HCV

Serum anti-HCV was assessed by an enzyme-linked immunosorbent assay (ELISA) diagnostic kit (Zhuohai Livzon Diagnostics Inc., China) according to the manufacturer's instructions. Quantitation of plasma HCV ribonucleic acid (RNA) was assessed using a 7500 real-time polymerase chain reaction (PCR) system (Applied Biosystems Inc., USA) by using an HCV RNA quantitative fluorescence diagnostic kit (Sansure Biotech Inc., China), and the lower detection limit was 25 IU/mL. All of the patients finally enrolled in the study were positive for serum anti-HCV antibodies and had detectable serum HCV RNA.

A multiplex one-step real-time fluorescent RT-PCR assay based on the analysis of the conserved sequence of HCV genome

was carried out in a SLAN-96P real-time fluorescence quantitative PCR instrument (Hongshi Medical Technology Co., Ltd, Shanghai, China) by using a hepatitis C virus genotype diagnostic kit (PCR-fluorescence probing) (Sansure biotech Inc., Hunan, China). This protocol can discern HCV genotypes 1–6 [17,18].

Statistical analysis

All statistical analyses were performed with IBM® SPSS® Statistics version 20.0, using descriptive statistical indexes such as mean, standard deviation (SD), median, and interquartile range (IRQ). For comparison of means or ratios, we performed the chi-squared test, analysis of variance (ANOVA), Kruskal-Wallis H test, LSD multiple comparisons tests, and Mann-Whitney U Multiple comparisons tests. The one-sample Kolmogorov-Smirnov test was used to verify the normal distribution of the datasets. The gender and age of the patients, the suspected initial infected time points, and transmission routes of the virus were included in the multinomial logistic regression analysis to predict the HCV GTs in the infected patients. The *P* values were all derived from double-tailed tests. A *P* value less than 0.05 was considered statistically significant.

Results

Distribution of the HCV genotypes (Table 1)

Among the 341 patients, genotype 1 (GT1) (72.1%) was the most common HCV genotype, followed by GT6 (17.6%), and GT3 (7.6%) and GT2 (2.6%). GT4 and GT5 were not found. The ages when HCV infections were confirmed in the patients infected with HCV GT3 or GT6 were significantly younger than those in the patients infected with HCV GT1, and their time intervals from suspected infection to confirmed clinical diagnosis made on visiting hospitals for HCV infection (TSICDs) were relatively shorter ($P < 0.05$). To some extent, TSICDs might represent the clinical latencies of the HCV-infected cases.

Suspected transmission routes of HCV

The suspected transmission routes of HCV were diverse (Tables 2, 3). Fourteen suspected transmission routes, including blood-related transmission, surgical operation, intravenous drug use (IDU), feculent sexual behavior, healthcare or non-healthcare invasive procedures, suspicious occupational exposure, or maternal-neonatal transmission, were mentioned in questionnaires by 301 patients (Table 2). Twenty-four patients gave a clear answer of 'unknown' for the question and 16 refused to answer. Twelve patients stated multiple suspected transmission routes in their questionnaires (Table 3). Blood-related transmission routes (57.5%) were the predominant transmission routes, followed by IDU (15.0%).

The above-mentioned 14 suspected transmission routes can be classified into 6 classes [2,3,6,7] (Table 4): blood-related routes, IDU and/or feculent sexual contact, healthcare invasive procedures, non-healthcare invasive procedures, others, and multiple transmission routes. We found that 93.1% of the patients infected through blood-related routes were infected with HCV GT1; 61.5% of the patients infected via IDU and/or feculent sexual contact were infected with HCV GT6; 71.4% of the patients infected through surgical operation without blood transfusion and other healthcare invasive procedures such as dental minor operation, injection, wound suture and hemodialysis were infected with HCV GT1; and 50.0% of the patients infected through non-healthcare invasive procedures such as tattooing, eyebrow embroidery, and ear piercing were infected with HCV GT6. The patients infected through blood-related routes or healthcare invasive procedures had a relatively longer duration from suspected infection to confirmed clinical diagnosis, so they were generally diagnosed at an older age. HCV infections via IDU or feculent sexual behavior were more prevalent in young males (88.5%), while infections via invasive cosmetic procedures occurred more in young females (83.3%), and both of them had a relatively shorter time interval from suspected infection to confirmed clinical diagnosis in hospitals: 9.2 ± 5.6 years and 2.3 ± 2.3 years, respectively vs. 18.1 ± 8.4 years in the whole group.

Distribution of virus transmission routes and HCV genotypes in patients with different suspected initial infection time points

There were 230 patients who could recall the suspected transmission routes of HCV and the time when the suspected initial HCV infections occurred.

Since July 1993, Chinese health management departments required blood donors be screened for HCV infection. The 'Blood Donation Law' has been implemented in mainland China since October 1998. Therefore, we classified the 230 cases into 3 groups according to the time when the suspected initial HCV infections occurred: before 1994, 1994–1998, and 1999–2016.

We found that that the number of HCV-infected patients in Hunan province continually decreased (Table 5, Figures 1, 2). However, younger patients infected with HCV, especially with HCV GT6 via IDU, feculent sexual behavior, or non-healthcare invasive procedures, significantly increased after 1999 (Tables 4, 5).

Multinomial logistic regression (Table 6)

A total of 225 infected cases were enrolled in the multinomial logistic regression analysis (Table 6). The likelihood ratio test indicated that the regression model was of significance

Table 1. Distribution of the HCV Genotypes in 341 HCV infected cases in Hunan Province, Investigated in 2016.

| HCV genotype | n (%) | Age diagnosed (year) ^c | | TSICD (year) ^d | |
|--------------|------------|-----------------------------------|---|---------------------------|-------------------|
| | | n | Median (25 th centile, 75 th centile) | n | Mean (SD) |
| 1 | 246 (72.1) | 235 | 47 (39, 56) | 178 | 19.5 (7.7) |
| 2 | 9 (2.6) | 9 | 54 (28.5, 62.5) | 7 | 24.7 (5.5) |
| 3 | 26 (7.6) | 23 | 36 (33, 39) | 14 | 10.4 (6.7) |
| 6 | 60 (17.6) | 60 | 36.5 (31, 42.8) | 23 | 9.4 (6.5) |
| Total | 341 | 327^a | 45 (35, 53.5) | 222^b | 18.1 (8.4) |

Age diagnosed – the age of the patient when the confirmed clinical diagnosis of HCV infection was made; SD – standard deviation; TSICD – time interval from suspected infection to confirmed clinical diagnosis made on visiting hospitals for HCV infection. a – 14 patients could not remember the time when they were diagnosed as HCV infected cases; b – 119 patients could not remember the time when they were diagnosed as HCV infected cases and/or the time when the suspected infection of the virus occurred; c – Age diagnosed: heterogeneity of variance, Kruskal-Wallis H test, $\chi^2=47.243$, $P=0.000$. Mann-Whitney U Multiple comparisons tests: GT1 vs. GT3 and GT1 vs. GT6, $P<0.05$; GT1 vs. GT2, GT3 vs. GT6, GT2 vs. GT3 and GT2 vs. GT6, $P>0.05$; d – TSICD: homogeneity of variance, $F=19.091$, $P=0.000$. LSD multiple comparisons tests: GT1 vs. GT3 GT1 vs. GT6, GT2 vs. GT3 and GT2 vs. GT6, $P<0.05$; GT1 vs. GT2 and GT3 vs. GT6, $P>0.05$.

Table 2. Distribution of the HCV genotypes in 341 hepatitis C patients in Hunan Province, investigated in 2016.

| Transmission route | Gender | | HCV genotypes, n (%) ^a | | | | Total |
|---|------------|------------|-----------------------------------|----------------|-----------------|------------------|------------|
| | M | F | 1 | 2 | 3 | 6 | |
| Blood donation | 8 | 27 | 35 (100) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 35 |
| Blood transfusion, excluding surgical operations with blood transfusion | 63 | 63 | 118 (93.7) | 3 (2.4) | 2 (1.6) | 3 (2.4) | 126 |
| Blood products transfusion | 3 | 0 | 2 (66.7) | 1 (33.3) | 0 (0.0) | 0 (0.0) | 3 |
| Dental minor operation, wound suture | 4 | 6 | 5 (50.0) | 0 (0.0) | 2 (20.0) | 3 (30.0) | 10 |
| Tattooing, eyebrow embroidery, ear piercing | 1 | 5 | 2 (33.3) | 0 (0.0) | 1 (16.7) | 3 (50.0) | 6 |
| Surgical operation without blood transfusion | 5 | 4 | 7 (77.8) | 1 (11.1) | 1 (11.1) | 0 (0.0) | 9 |
| Surgical operation with blood transfusion | 22 | 18 | 35 (87.5) | 2 (5.0) | 1 (2.5) | 2 (5.0) | 40 |
| IDU | 40 | 4 | 5 (11.4) | 0 (0.0) | 12 (27.3) | 27 (61.4) | 44 |
| Feculent sexual behavior | 3 | 2 | 1 (20.0) | 0 (0.0) | 0 (0.0) | 4 (80.0) | 5 |
| Needle sharing and/or no sterilizing of injector (excluding IDU) | 3 | 4 | 7 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 7 |
| Hemodialysis | 0 | 1 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) | 1 |
| Occupational exposure | 1 | 1 | 1 (50.0) | 0 (0.0) | 0 (0.0) | 1 (50.0) | 2 |
| Maternal-neonatal transmission | 0 | 1 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) | 1 |
| Multiple suspected transmission routes | 8 | 4 | 6 (50.0) | 0 (0.0) | 2 (16.7) | 4 (33.3) | 12 |
| Unclear | 11 | 13 | 13 (54.2) | 2 (8.3) | 2 (8.3) | 7 (29.2) | 24 |
| Unwilling to answer | 8 | 8 | 9 (56.2) | 0 (0.0) | 3 (18.8) | 4 (25.0) | 16 |
| Total | 180 | 161 | 246 (72.1) | 9 (2.6) | 26 (7.6) | 60 (17.6) | 341 |

F – female; IDU – intravenous drug use; M – male; Unclear – with definite answer ‘unclear transmission route’. a – Distribution of the HCV genotypes: Pearson $\chi^2=208.506$, $P=0.000$

Table 3. Data of the 12 patients infected with HCV through multi-transmission routes, Hunan Province, Investigated in 2016.

| No. | Gender | Age (year) | HCV genotype | Age diagnosed (year) | Suspected transmission routes |
|-----|--------|------------|--------------|----------------------|--|
| 1 | M | 47 | 1 | 47 | IDU, feculent sexual behavior |
| 2 | M | 39 | 3 | 23 | IDU, feculent sexual behavior |
| 3 | F | 36 | 1 | 31 | Blood transfusion, maternal-neonatal transmission |
| 4 | F | 59 | 1 | 57 | Blood products transfusion, dental minor operation |
| 5 | M | 38 | 6 | 33 | Surgical operations with blood transfusion, IDU |
| 6 | M | 32 | 6 | 30 | IDU, tattooing, feculent sexual behavior |
| 7 | M | 37 | 3 | 37 | IDU, tattooing |
| 8 | F | 45 | 1 | 35 | Surgical operations with blood transfusion, Cosmetic surgery |
| 9 | F | 28 | 1 | 27 | Dental minor operation, blood donation |
| 10 | M | 43 | 6 | 40 | IDU, feculent sexual behavior |
| 11 | M | 56 | 1 | 45 | Hemodialysis, renal transplantation |
| 12 | M | 27 | 6 | 27 | IDU, blood transfusion |

Age diagnosed – the age of the patient when the confirmed clinical diagnosis of HCV infection was made; F – female; IDU – intravenous drug use; M – male.

($\chi^2=130.039$, $P<0.01$). The suspected initial infected time points and transmission routes of the virus were significantly correlated with the distribution of HCV GTs (χ^2 23.528 and 38.399, respectively, both $P<0.01$). Estimation of parameters showed that for the cases infected before 1994, HCV GT1 infection was more prevalent than HCV GT3 and HCV GT6 infection (χ^2 5.445 and 6.434, respectively, both $P<0.05$), while for the cases infected via IDU or feculent sexual behavior transmission routes, HCV GT3 and HCV GT6 infection was more prevalent than HCV GT1 infection (χ^2 6.383 and 5.525, respectively, both $P<0.05$), and for the cases infected via illegal blood donation or blood or blood products transfusion transmission routes, HCV GT1 infection was more prevalent than HCV GT6 infection ($\chi^2=5.155$, $P<0.05$).

Discussion

Because current HCV vaccine trials are still in the early phases and progressing slowly [19], the current strategies for HCV prevalence prevention are to reduce transmission by reducing the risk of exposure to the virus or to cure the HCV-infected patients with effective treatment such as interferon (IFN)-based therapy or newly developed direct-acting antiviral agents (DAAs). However, the IFN-based therapy and currently available DAAs are both HCV GT-specific [5,15,16], and HCV GT distribution patterns are significantly different in patients infected with HCV via various transmission routes [1–3,6,9].

Therefore, it is important to understand the patterns of variation of different transmission routes based on virus genotyping so as to formulate reasonable strategies for HCV prevention and control.

HCV GT distribution patterns in various Chinese regions are experiencing a remarkable change with increasing population mobility, variation of transmission routes, and improvement of testing methods [6,8,10–13]. The dominant HCV GTs in most provinces are GT1 and GT2, while the regions in southern China have an abundant HCV genetic diversity in which HCV GT3 and GT6 are more prevalent [10–13,20–23]. Our data revealed that HCV genetic diversity in Hunan province was mainly related to the variation of transmission routes in different periods.

Although it is difficult to accurately calculate the incubation period of the initial HCV infection due to the slow progression of most chronic HCV-infected cases, the estimated time points of the initial infections based on the patients' recall of their first contact with the virus via suspected transmission routes are acceptable. Our data revealed that most of the HCV-infected patients in Hunan province were infected via blood-related routes before 1999, especially before July 1993 when Chinese health management departments required blood donors be screened for HCV infection. Since then, the number of HCV-infected patients has continually decreased, mainly due to the standardized management of blood and blood products. Similar to the other provinces of mainland China

Table 4. Distribution of HCV genotypes in patients infected through different classified transmission routes in 341 hepatitis C patients in Hunan Province, investigated in 2016.

| Group | Transmission route | Gender ^c | | HCV genotype, n (%) ^d | | | | Age diagnosed (year) Median (25 th centile, 75 th centile) | TSICD (year) Mean (SD) |
|-------|---------------------------|---------------------|-----|----------------------------------|------------|--------------|--------------|--|---------------------------|
| | | M | F | 1 | 2 | 3 | 6 | | |
| R1 | Blood | 96 | 108 | 190 (93.1) | 6 (2.9) | 3 (1.5) | 5 (2.5) | 48.0 (39.0, 57.0) | 20.0 (7.7) |
| R2 | IDU/Sex | 46 | 6 | 7 (13.5) | 0 (0.0) | 13 (25.0) | 32 (61.5) | 38.0 (32.5, 40.5) | 9.7 (5.6) |
| R3 | Healthcare invasive | 13 | 15 | 20 (71.4) | 1 (3.6) | 3 (10.7) | 4 (14.3) | 46.0 (34.5, 54.5) | 13.5 (8.6) |
| R4 | Non-healthcare invasive | 1 | 5 | 2 (33.3) | 0 (0.0) | 1 (16.7) | 3 (50.0) | 27.0 (23.0, 32.3) | 2.3 (2.3) |
| R5 | Other | 1 | 2 | 1 (33.3) | 0 (0.0) | 0 (0.0) | 2 (66.7) | a | b |
| R6 | Multi-transmission routes | 4 | 4 | 4 (50.0) | 0 (0.0) | 1 (12.5) | 3 (37.5) | 32.0 (27.8, 36.5) | 12.8 (2.6) |
| Total | | 161 | 140 | 224 (74.4) | 7 (2.3) | 21 (97) | 49 (16.3) | 45.0 (35.0, 53.5) | 18.1 (8.4) |

Age diagnosed – the age of the patient when the confirmed clinical diagnosis of HCV infection was made; Blood – blood related routes, including blood donation, blood or blood products transfusion, including surgical operations with blood transfusion; F – female; Healthcare invasive – surgical operation without blood transfusion and other healthcare invasive procedures, including dental minor operation, needle sharing and/or no sterilizing of injector (excluding IDU), wound suture and hemodialysis; IDU/Sex – intravenous drug use, and/or feculent sexual behavior; M – male; Non-healthcare invasive – non-healthcare invasive procedures, such as tattooing, eyebrow embroidery, ear piercing; Other – one of the other transmission routes; SD – standard deviation; TSICD – time interval from suspected infection to confirmed clinical diagnosis made on visiting hospitals for infection of HCV. a – The ages diagnosed of the three patients infected via other route (R5) were 3, 41 and 58 years, respectively; b – TSICDs of the three patients infected via other route (R5) could not be calculated because the time points of the suspected initial HCV infections were unclear; c – Gender: Pearson $\chi^2=33.315$, $P=0.000$; d – HCV genotype: Pearson $\chi^2=175.958$, $P=0.000$; e – age diagnosed: heterogeneity of variance, Kruskal-Wallis H test, $\chi^2=46.269$, $P=0.000$. Mann-Whitney U Multiple comparisons tests: R1 vs. R2, R1 vs. R4, R1 vs. R6, R2 vs. R3, R2 vs. R4, R3 vs. R4 and R3 vs. R6, $P<0.05$; R1 vs. R3, R1 vs. R5, R2 vs. R6, R3 vs. R5, R4 vs. R5, R4 vs. R6 and R5 vs. R6, $P>0.05$. f – TSICD: homogeneity of variance, $F=15.977$, $P=0.000$. LSD multiple comparisons tests: R1 vs. R2, R1 vs. R3, R1 vs. R4, R1 vs. R6, R3 vs. R4, R2 vs. R4 and R3 vs. R4, $P<0.05$; R2 vs. R3, R2 vs. R4, R2 vs. R6, R3 vs. R6 and R4 vs. R6, $P>0.05$.

[6–8,23–26], blood-related routes of transmission of HCV GT1 are the most common.

Hunan Province has a large population of more than 65 million and it is not feasible to screen HCV infectors in the whole population. Because of the limited economic capabilities and medical conditions, many potential patients have not visited any medical institution for HCV infection screen since the years when the high-risk blood-related exposures occurred. On the other hand, the characteristics of long-term clinically silent and progressive aggravation of many HCV infections might lead to lose of opportunities for timely treatments in many potential patients [6]. Here, we can conclude that it is essential to screen for HCV infection in the groups with history of blood donation or of blood or blood product transfusion before 1999, especially before 1994. In this way, the majority of the potential HCV-infected people can be detected, which

would be of great significance to epidemic control and prevention, as well as treatment of hepatitis C in the province.

With the changes in social and economic conditions, patients infected with HCV, especially with HCV GT6 via IDU, significantly increased in recent years in China [6,7,12,14,22]. Our data revealed that both the absolute number and the proportion of HCV GT6-infected cases are increasing in recent years in Hunan province, and a large proportion of them are young male IDUs, similar to many southern and southwestern provinces in mainland China [6–8,12,14,22,27]. More attention should be paid to these young IDUs who may have become the most important routes of potential HCV transmission link in Hunan province. Although the incidence of sexually transmitted HCV infection remains low in mainland China [27,28], several cases believed to be infected with HCV transmitted via feculent sexual behaviors were investigated in our study and most of

Table 5. Distribution of virus transmission routes and HCV genotypes in 230 patients with clear suspected initial infection time points in Hunan Province, investigated in 2016.

| Year of Infection | HCV transmission routes, n (%) ^b | | | | | HCV genotype, n (%) ^c | | | | |
|-------------------|---|-----------|---------------------|-------------------------|------------------------|----------------------------------|---------|-----------|-----------|------------------------|
| | Blood | IDU/Sex | Healthcare invasive | Non-healthcare invasive | Total | 1 | 2 | 3 | 6 | Total |
| Before 1994 | 114 (94.2) | 1(0.8) | 5 (4.1) | 1 (0.8) | 121 (100) | 111 (92.5) | 7 (5.8) | 1 (0.8) | 1 (0.8) | 120 (100) |
| 1994–1998 | 51 (86.4) | 5 (8.5) | 3 (5.1) | 0 | 59 (100) | 52 (88.1) | 0 | 2 (3.5) | 5 (8.5) | 59 (100) |
| 1999–2016 | 22 (40.0) | 22 (40.0) | 7 (12.7) | 4 (7.3) | 55 (100) | 22 (43.1) | 0 | 11 (21.6) | 18 (35.3) | 51 (100) |
| Total | 187 (79.6) | 28 (11.9) | 15 (6.4) | 5 (2.1) | 235 (100) ^a | 185 (80.4) | 7 (3.0) | 14 (6.1) | 24 (10.5) | 230 (100) ^a |

Blood – blood related routes; Healthcare invasive – surgical operation without blood transfusion and other healthcare invasive procedures, including dental minor operation, wound suture and hemodialysis; IDU/Sex – intravenous drug use, and/or feculent sexual behavior; Non-healthcare invasive – non-healthcare invasive procedures, such as tattooing, eyebrow embroidery, ear piercing. a – 5 of the 230 cases provided two kinds of suspected HCV transmission routes; b – HCV transmission routes, chi-squared tests: Pearson $\chi^2=79.882, P=0.000$. Blood, Pearson $\chi^2=116.042, P=0.000$; IDU/Sex, Pearson $\chi^2=56.176, P=0.000$; Healthcare invasive, Pearson $\chi^2=4.897, P=0.086$; Non-healthcare invasive, continuity corrected chi square $\chi^2=6.187, P=0.013$; c – HCV genotype, chi-squared tests: Pearson $\chi^2=85.095, P=0.000$.

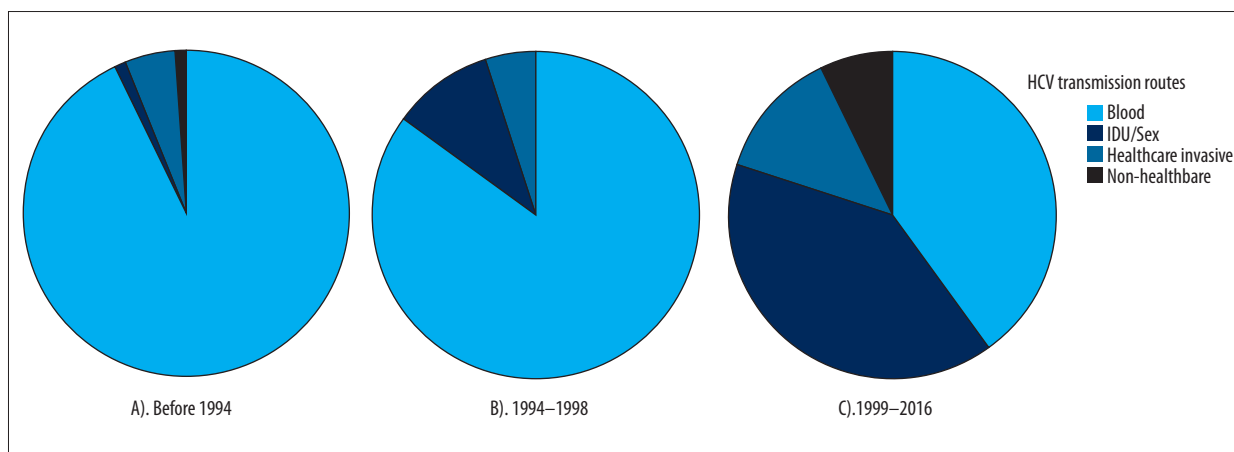


Figure 1. Distribution of virus transmission routes in 230 HCV-infected cases with clear suspected initial infection time points in Hunan province, investigated in 2016. Blood, blood-related routes, including blood donation, blood or blood products transfusion, including surgical operations with blood transfusion; Healthcare invasive, surgical operation without blood transfusion and other healthcare invasive procedures, including dental minor operation, needle sharing and/or no sterilizing of injector (excluding IDU), wound suture and hemodialysis; IDU/Sex – intravenous drug use, and/or feculent sexual behavior; M – male; Non-healthcare invasive, non-healthcare invasive procedures, such as tattooing, eyebrow embroidery, ear piercing.

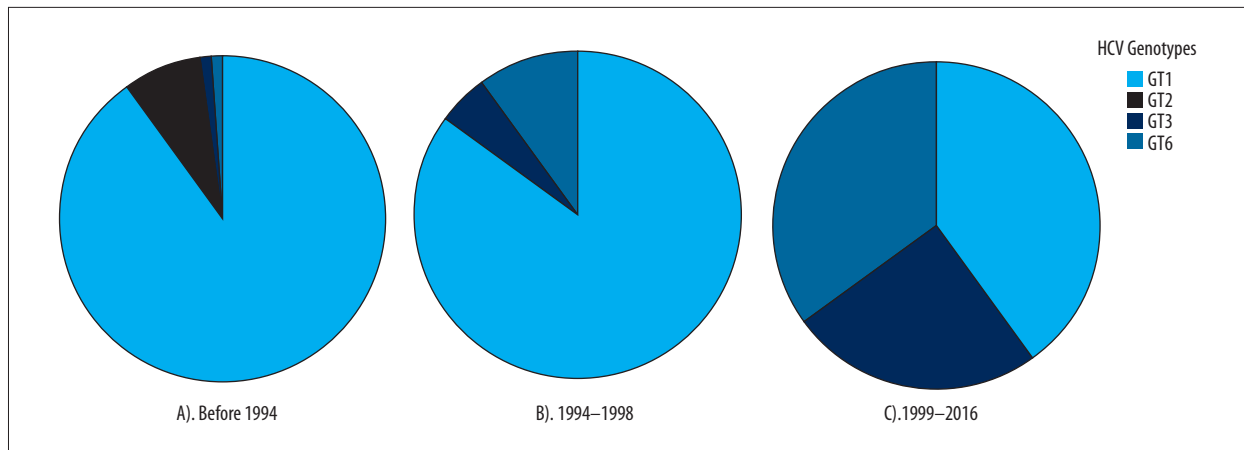


Figure 2. Distribution of HCV genotypes in 230 HCV-infected cases with clear suspected initial infection time periods in Hunan province, investigated in 2016.

Table 6. Multinomial logistic regression analysis of the predictors of HCV GT3 and GT6 compared to GT1 in the HCV infected patients in Hunan Province, 2016.

| HCV GT ^a | Variable | Beta value | Wald chi-square | P value | OR (95%CI) | |
|------------------------|------------------------|-------------|-----------------|---------|----------------------|------------------------|
| HCV GT3 | Intercept | -0.864 | 0.681 | 0.409 | | |
| | Gender | -2.175 | 3.164 | 0.075 | 0.114 (0.010-1.248) | |
| | Age (≥40 years or not) | 1.543 | 3.343 | 0.068 | 4.677 (0.895-24.440) | |
| | Year of infection | Before 1994 | -2.837 | 5.445 | 0.020 | 0.059 (0.005-0.635) |
| | | 1994-1998 | -2.130 | 3.076 | 0.079 | 0.119 (0.011-1.284) |
| | | 1999-2016 | 0 | | | |
| | Transmitting routes | Blood | -1.068 | 0.847 | 0.357 | 0.344 (0.035-3.338) |
| | | IDU/sex | 3.760 | 6.383 | .012 | 42.931 (2.323-793.317) |
| | | Other | 0 | | | |
| | HCV GT6 | Intercept | -0.255 | 0.089 | 0.765 | |
| [Gender=1] | | -0.092 | 0.014 | .907 | 0.913 (0.195-4.265) | |
| Age (≥40 years or not) | | 0.255 | 0.110 | 0.740 | 1.290 (0.286-5.818) | |
| Year of infection | | Before 1994 | -2.954 | 6.434 | 0.011 | 0.052 (0.005-0.511) |
| | | 1994-1998 | -1.070 | 1.960 | 0.161 | 0.343 (0.077-1.534) |
| | | 1999-2016 | 0 | | | |
| Transmitting routes | | Blood | -1.981 | 5.155 | 0.023 | 0.138 (0.025-0.763) |
| | | IDU/sex | 2.341 | 5.525 | 0.019 | 10.388 (1.475-73.149) |
| | | Other | 0 | | | |

HCV – hepatitis C virus; GT – genotype; OR – odds ratio; CI – confidence interval; Blood – blood related routes; IDU/Sex – intravenous drug use, and/or feculent sexual behavior. a – HCV GT1 was grouped in the reference category.

them were infected with HCV GT6. It is important to promote a healthy lifestyle in young men.

Non-healthcare invasive procedures in private cosmetic stores and minor dental procedures, wound suturing or acupuncture in sub-standard small clinics where needle- or syringe-sharing

are not yet fully eliminated, expose the customers to higher risk of HCV transmission [7,8,29]. Our data revealed that the HCV GT distribution of patients infected via such transmission routes in Hunan province was diverse, with GT6 as the most prevalent genotype, perhaps because HCV GT6 infection is more common in young people who prefer to pursue fashion

and personalized experience. Legal management of the proliferation of private cosmetic stores without standardized procedures or formally trained professionals is an emergent and arduous task for HCV prevention and control in Hunan province.

Based upon the findings of our study, the key points for prevention and control of HCV infection in Hunan province are screening for HCV infection among people who received a blood transfusion or were blood donors before 1999, management of the small businesses such as private dental clinics and cosmetic stores, and control of drug abuse. If HCV genotyping cannot be implemented in some patients due to the limited resources in the province, anti-HCV GT1 therapeutic regimens can be recommended to the cases infected via blood-related routes, while recommending anti-HCV GT1, GT3, and GT6 regimens to the cases infected via other routes.

Although the research subjects in this study were enrolled from one of the 2 largest hospitals treating hepatitis patients in Hunan province and all were from the 14 districts of the province, the possibility of selection bias cannot be ignored. The tentative findings in our study call for a further research in a larger cohort.

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Conclusions

HCV GT1 infections via blood-related transmission routes in Hunan province have continually decreased since 1994. However, younger patients infected with HCV, especially with HCV GT6 via IDU, feculent sexual behavior, and non-health-care invasive procedures, have significantly increased. Thus, it is important in Hunan province to screen for HCV infection in groups with history of blood donation or transfusion before 1999, to manage small businesses such as private dental or cosmetic clinics, and to control IDU. The correlations between HCV genotypes and transmission routes can be used to guide the choice of therapeutic regimens.

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Conflict of interests

None.

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