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Correlation Analysis of HCV Genotyping with Viral load, Liver Function, and Liver Fibrosis in Anti-HCV Positive Patients in Yueyang Area

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Abstract:

Objective: To analyze the distribution of HCV genotypes among anti-HCV-positive patients in Yueyang using next-generation sequencing (NGS), and to examine their associations with sex, age, viral load, liver function, and liver fibrosis markers.

Methods: A total of 133 anti-HCV-positive patients admitted to Yueyang People's Hospital from October 2024 to January 2026 were included. Demographic data, HCV-RNA viral load, HCV genotyping results, liver function indicators, and liver fibrosis markers were collected. Genotype distribution, NGS detection performance, and differences in clinical indicators among genotypes were analyzed.

Results: Five HCV genotypes were identified among the 133 patients. Genotype 1b was predominant, accounting for 42.9%, followed by 6a at 26.3%, 3a at 10.5%, mixed genotype infection at 4.5%, and 2a at 2.3%. Sex distribution did not differ significantly among genotypes ($P>0.05$). Age distribution showed an overall significant difference ($P<0.05$), but no between-group difference remained significant after correction for multiple comparisons. No significant differences were found among genotypes in HCV viral load, ALT, AST, ALP, GGT, TBIL, TBA, HA, CIV, PIIIP, or LN ($P>0.05$), while DBIL differed significantly ($P<0.05$). The positive agreement rate, negative agreement rate, and overall agreement rate of NGS for HCV genotyping were all 100.0%, with a Kappa value of 1.000.

Conclusion: HCV genotype 1b was the predominant genotype in Yueyang, followed by 6a. Most clinical indicators did not differ significantly among genotypes, except for DBIL. The FASTASeq 300 Dx gene sequencer showed high accuracy in HCV genotyping and may provide reliable evidence for individualized treatment of hepatitis C.

Keywords: Chronic hepatitis C; Genotype; Viral load; Liver function; Liver fibrosis

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Introduction

Hepatitis C virus (HCV) is a single-stranded RNA virus belonging to the Flaviviridae family. It has a positive liver effect and can cause acute and chronic hepatitis. Chronic hepatitis C infection with progressive liver damage may lead to cirrhosis and related complications, including compensated liver disease and hepatocellular carcinoma [1]. According to the World Health Organization, in 2015, there were 71 million people with chronic HCV infection worldwide, and 399,000 people died from cirrhosis or hepatocellular carcinoma (HCC) caused by HCV infection. In 2019, there were 58 million people with chronic HCV infection worldwide, and 290,000 people died from cirrhosis or HCC caused by HCV infection [2]. Therefore, early diagnosis and treatment of HCV infection are extremely important. There are about 5.6 million people with HCV infection in the general population in China. If we include HCV infection in high-risk groups and high-incidence areas, the number is estimated to be about 10 million [2]. HCV has high genetic heterogeneity, and at least 7 genotypes and multiple subtypes have been

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discovered to date. The distribution of different genotypes varies significantly across the globe and is closely related to antiviral treatment response, disease progression and prognosis [3]. For example, genotype 1b has a relatively low response rate to direct-acting antiviral drugs (DAAs), while genotype 3, especially genotype 3a, is associated with accelerated progression of liver fibrosis [4]. Therefore, accurate HCV genotyping results are a key prerequisite for developing individualized treatment plans and improving treatment success rates. At the same time, the HCV RNA viral load level can directly reflect the degree of viral replication activity and is closely related to hepatocellular damage, liver inflammation and fibrosis process. It is a core indicator for laboratory assessment of disease activity. Traditional HCV genotyping methods include PCR-restriction fragment length polymorphism analysis, sequencing, etc., but they have shortcomings such as low throughput and limited resolution [5]. Next-Generation Sequencing (NGS) technology has been gradually applied to viral genotyping and mutation analysis due to its advantages of high throughput, high accuracy and single-base resolution [6]. The FASTASeq 300 Dx gene sequencer uses Sequencing By Synthesis (SBS) technology to achieve efficient and accurate nucleic acid sequence determination. Based on this sequencing platform, this study analyzed the genotypes of 133 HCV antibody-positive patients in Yueyang area, aiming to clarify the distribution characteristics of HCV genotypes in the region and explore their relationship with viral load, liver function and liver fibrosis detection results. At the same time, it verified the application value of NGS technology in clinical HCV genotyping detection and provided a scientific basis for regional hepatitis C prevention and control and precision treatment.

Material and Methods

General Information

This study included 133 patients with positive anti-HCV antibodies admitted to Yueyang People's Hospital between October 2024 and January 2026. The study population consisted of 76 males (57.1%) and 55 females (41.4%), with an age range of 29–83 years and a mean age of (56.2±11.1) years, predominantly middle-aged individuals. Based on HCV-RNA viral load detection results, patients were divided into high viral load group (>10^6 IU /ml), medium viral load group (10^4–10^6 IU /ml), low viral load group (<10^4 IU /ml), and undetectable group. The high viral load group comprised 88 cases (66.2%), the medium viral load group 24 cases (18.0%), the low viral load group 6 cases (4.5%), and the undetectable group 15 cases (11.3%), indicating that viral replication was active in most patients. A retrospective analysis was conducted on the patients' general clinical data, genotyping results, liver function test results, and liver fibrosis test results.

Inclusion criteria

1) Positive HCV antibody test; **2)** Willing to participate in this study and sign informed consent form; **3)** Complete clinical data.

Exclusion criteria

1) Insufficient sample size or failure to collect and preserve samples as required; **2)** Missing information that cannot be traced; **3)** Duplicate samples from the same patient; **4)** Other circumstances deemed unsuitable for inclusion by the investigator.

This study was approved by the Ethics Review Committee for Clinical Trials of Drugs/Medical Devices of Yueyang People's Hospital (Ethics Approval No.: 2024040) and was conducted in strict accordance with the requirements of the Good Clinical Practice for Medical Devices (No. 28 of 2022).

Methods

HCV genotyping

1) Next-Generation Sequencing (NGS) Technology: FASTASeq 300 Dx gene sequencer (Shenzhen Zhenmai Biotechnology Co., Ltd.), along with a matching Hepatitis C Virus Genotyping Kit (reversible terminator sequencing method) and a universal sequencing reaction kit; **2)** Comparison Reagent: Hepatitis C Virus (HCV) Genotyping Kit (PCR-fluorescent probe method, Taipu Bioscience (China) Co., Ltd., National Medical Device Registration Certificate No. 20143401926); **3)** Verification Method: Sanger sequencing kit (Applied Biosystems , USA); **4)** Nucleic Acid Extraction Reagent: Nucleic acid extraction reagent (Minxia Medical Device Registration No. 20150045) and QIAamp Viral RNA Mini Kit (Qiagen , Germany , Catalog No.: 52904); **5)** Detection Steps: Sample Collection and Processing: Collect 5 mL of venous blood from the subject, centrifuge to separate the serum, and store as required (store at 2~8℃ for no more than 72 hours, at -25~-15℃ for no more than 3 months , and below -70℃ for no more than 1 year). Avoid repeated freeze-thaw cycles and bring to room temperature before testing. Nucleic Acid Extraction: Use the nucleic acid extraction reagents for the experimental group and control group respectively , and extract nucleic acid from the serum samples according to the instructions. The sample input for the experimental group is 200 μL of serum, and the initial sample input for the control group is 200 μL of serum. Detection Procedure: 1. Experimental Group: Take 15 μL of the extracted product for PCR amplification and library construction (PCR amplification product concentration ≥10 ng/μL). Add the 4 nM library to the sequencer, set the sequencing read length PE150, the cycle number of strand 1 and strand 2 to 150, and the tag sequence length to 8+8, and perform sequencing detection. The required sequencing data volume is ≥0.5 Gb, the Q30 base ratio is ≥80%, and the sequence alignment to the reference genome ratio is ≥90%. 2. Control Group: 10 μL of the extracted product was used for PCR-fluorescent probe detection. Reaction parameters were strictly set according to the kit instructions. Negative control solutions showed no typical S-type amplification curve or a ct

Table 1 | liver function biomarkers

Biomarker	Method Principle
Alanine aminotransferase (ALT)	IFCC enzymatic rate method (kinetic UV assay, pyridoxal-5′-phosphate activated)
Aspartate aminotransferase (AST)	IFCC enzymatic rate method (kinetic UV assay)
Alkaline phosphatase (ALP)	Kinetic colorimetric method (p-nitrophenyl phosphate substrate)
γ-Glutamyl transferase (GGT)	Enzymatic rate method (γ-glutamyl-3-carboxy-4-nitroanilide substrate)
Total bilirubin (TBIL)	Colorimetric method (dichlorophenyldiazonium, DPD, or vanadate oxidation method)
Direct bilirubin (DBIL)	Colorimetric method (DPD diazo method or bilirubin oxidase method)
Albumin (ALB)	Colorimetric method (bromocresol green, BCG)
Total protein (TP)	Colorimetric method (biuret reaction)

value > 26.5. Positive control HCV 1b PCR reaction tubes showed a typical S-type FAM-labeled curve with a ct value ≤ 25.1. Other reaction solutions showed no typical S-type amplification curve or a ct value > 26.5. Otherwise, the experiment was invalid and needed to be repeated. 3. Result Verification: Samples with inconsistent results from the two groups were verified using Sanger sequencing. Positive Criterion: A genotype is considered positive if the specific read length ratio (RPM) per million sequences is ≥ 119778.

HCV RNA detection

HCV RNA quantification was performed using the Sansure High-Sensitivity HCV RNA Quantitative Detection Kit (PCR-Fluorescence Probing Method; Sansure Biotech, Changsha, China). Nucleic acid extraction was conducted manually using a superparamagnetic nano-bead-based method according to the manufacturer's protocol. Real-time PCR amplification and detection were carried out on compatible thermocyclers, including the SLAN-96P (Hongshi) and ABI 7500 (Thermo Fisher Scientific).

The assay is based on a one-step reverse transcription real-time quantitative PCR (RT-qPCR) using fluorescence-labeled probes targeting conserved regions of the HCV genome. Briefly, 0.2 mL of serum or plasma was subjected to room-temperature chemical lysis without heating, followed by RNA capture using nano-core-shell magnetic beads (nanoscale magnetic core with a molecular polymer shell) to efficiently enrich and purify nucleic acids. The extracted RNA was then added to the PCR reaction mixture containing specific primers, a fluorescence-labeled probe, heat-activated DNA polymerase, deoxynucleotide triphosphates (dNTPs), and an internal control (IC) included throughout the extraction and amplification process to monitor PCR inhibition and prevent false-negative results. The reaction system also contained ROX passive reference dye to normalize well-to-well variations and pipetting errors, thereby improving quantitative accuracy.

HCV antibody detection

HCV antibody screening was performed using the AiD™ anti-HCV ELISA kit (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China). The assay was read on a KHB ST-360 microplate reader (Shanghai Kehua Experimental System Co., Ltd., Shanghai, China), and plate washing was performed using a KHB ST-36W microplate washer (Shanghai Kehua). All reagents were used within their stated shelf life.

The assay is based on a third-generation enzyme-linked immunosorbent assay (ELISA). HCV recombinant antigens (Core, NS3, NS4, and NS5 regions) are pre-coated onto polystyrene microwells. Serum or plasma samples (typically 100 µL) are added to the antigen-coated wells and incubated. Anti-HCV antibodies, if present, bind to the immobilized antigens. After washing to remove unbound material, horseradish peroxidase (HRP)-conjugated anti-human IgG is added, which binds to any antigen–antibody complexes formed. Following a second wash, the 3,3′,5,5′-tetramethylbenzidine (TMB) chromogenic substrate is added and hydrolyzed by the bound HRP to produce a blue-colored product. The reaction is terminated by adding stop solution, and the optical density (OD) is measured at 450 nm (with a reference wavelength of 630 nm) on the KHB ST-360 reader. The intensity of the color is proportional to the concentration of anti-HCV antibodies in the sample.

Liver function biochemical index testing

Liver function tests were performed on the DxA 5000 Fit total laboratory automation system (Beckman Coulter, Brea, CA, USA), which integrates pre-analytical sample processing with connected clinical chemistry and immunoassay analyzers. The connected chemistry analyzers (e.g., UniCel DxC or AU5800 series, Beckman Coulter) were operated with Beckman Coulter original reagents according to the manufacturer's instructions. All assays were conducted under standardized laboratory conditions with routine calibration and quality control procedures.

The liver function biomarkers were quantified in serum or plasma are listed in **Table 1**.

Liver fibrosis marker detection

Serum liver fibrosis markers were quantified on the MAGLUMI X8 fully automated chemiluminescence immunoassay (CLIA) analyzer (Shenzhen New Industry Biomedical Engineering Co., Ltd. [Snibe], Shenzhen, China). The system was operated with Snibe original reagents, including the following assay kits: Hyaluronic Acid (HA) assay kit, Laminin (LN) assay kit, Type IV Collagen (CIV) assay kit, and Type III Procollagen N-terminal Peptide (PIIINP) assay kit. All reagents and calibrators were used within their stated

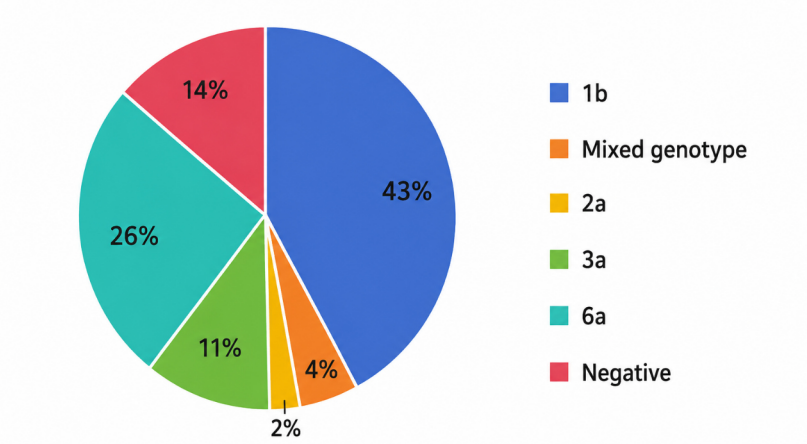

Figure 1 | Distribution of HCV genotypes

shelf life and stored at 2–8℃ according to the manufacturer's instructions.

The assay is based on a direct chemiluminescence immunoassay (CLIA) with magnetic microparticle separation. Briefly, serum samples (typically 20–50 μL, depending on the specific marker) are incubated with nano-magnetic bead–coated capture antibodies (or antigens, depending on the assay format) and acridinium ester–labeled detection antibodies. The antigen–antibody complexes are captured on the magnetic microparticles, and unbound substances are removed by a magnetic separation and wash step. Subsequently, a trigger solution is added to initiate the chemiluminescent reaction of the acridinium ester label, and the emitted light is measured in relative light units (RLU) by the photomultiplier tube of the MAGLUMI X8. The RLU value is proportional to the concentration of the target analyte in the sample. Quantification is achieved by interpolation from a master calibration curve stored on board the analyzer, which is recalibrated using two-point calibration with Snibe calibrators according to the manufacturer's protocol.

Statistical analysis

SPSS 27.0 software was used for statistical analysis. First, the normality of the measurement data was tested. The measurement data in this study did not conform to the normal distribution after the normality test, and were expressed as medians (interquartile range) [M (P25 ~ P75)]. The Kruskal -Wallis test was used for comparisons between groups , and the χ^2 test was used for count data. A $p < 0.05$ was considered statistically significant.

Results

HCV genotype distribution

Anti -HCV patients included: genotype 1b in 57 cases (42.9%), genotype 2a in 3 cases (2.3%), genotype 6a in 35 cases (26.3%), genotype 3a in 14 cases (10.5%), and mixed genotype in 6 cases (4.5%). See Figure 1.

Correlation analysis of HCV genotype with sex and age

In this study, there were 34 males (60.0%) and 23 females (40%) with genotype 1b; 1 male (33%) and 2 females (66%) with genotype 2a; 8 males (57%) and 6 females (43%) with genotype 3a; 5 males (83%) and 1 female (17%) with mixed genotype; and 20 males (57%) and 15 females (43%) with genotype 6a. Sex comparisons were performed among the patients with the five genotypes. The results showed that, according to the chi-square test, there was no statistically significant difference in the gender distribution of patients with different HCV genotypes (χ^2=4.461, P=0.669 $>$0.05); according to the Kruskal -Wallis H test, there was a statistically significant difference in the age distribution of patients with different HCV genotypes (χ^2=9.877, df =4, P=0.043<0.05), with the highest median age for genotype 2a (65 years) and the lowest for genotype 6a (53 years). However, after post-hoc multiple comparison correction, there was no statistically significant difference in age among the genotype groups (P>0.05). See Table 2 and Figure 2.

Correlation analysis between HCV genotype and viral load

HCV RNA is a direct marker of viral replication and an important laboratory basis for confirming HCV infection in clinical practice. It is also a standard for evaluating the efficacy of antiviral treatment. Therefore, exploring the relationship between HCV genotype and viral load is of great significance for clinical diagnosis and treatment. In this study, the median HCV RNA viral load for genotype 1b was 3.28×10^6 IU/mL, for genotype 2a it was 2.20×10^4 IU / mL , for genotype 3a it was 5.14×10^6 IU/ mL , for genotype 6a it was 5.02×10^6 IU/ mL , and for mixed genotype HCV RNA it was 3.24×10^6 IU / mL . Comparison of viral load among different genotypes showed no statistically significant difference (P > 0.05). See Table 3 and Figure 3.

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Table 2 | Sex distribution of different genotypes

Genotype	Male (n%)	Female (n%)
1b	34 (60%)	23 (40%)
2a	1 (33%)	2 (66%)
3a	8 (57%)	6 (43%)
Hybrid	5 (83%)	1 (17%)
6a	20 (57%)	15 (43%)

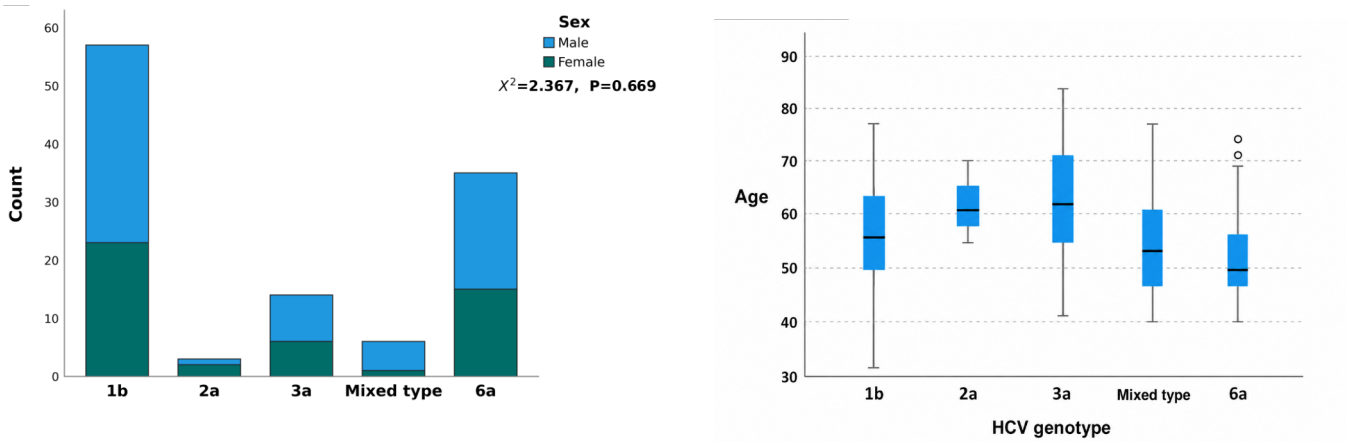

Figure 2 | Comparison of different HCV genotypes by age and sex

Table 3 | Comparison of viral load of different HCV genotypes [M(P25~P75)]

HCV genotype	Number of examples	Viral load (IU/ mL)
1b	57	3.28×10^6 (1.21×10^6 ~ 1.38×10^7)
2a	3	2.20×10^4 (1.71×10^4 ~ 7.94×10^4)
3a	14	5.14×10^6 (3.86×10^5 ~ 9.23×10^6)
Hybrid	6	3.24×10^6 (5.33×10^5 ~ 6.60×10^6)
6a	35	5.02×10^6 (1.23×10^6 ~ 2.35×10^7)
H value		9.306
p -value		0.054

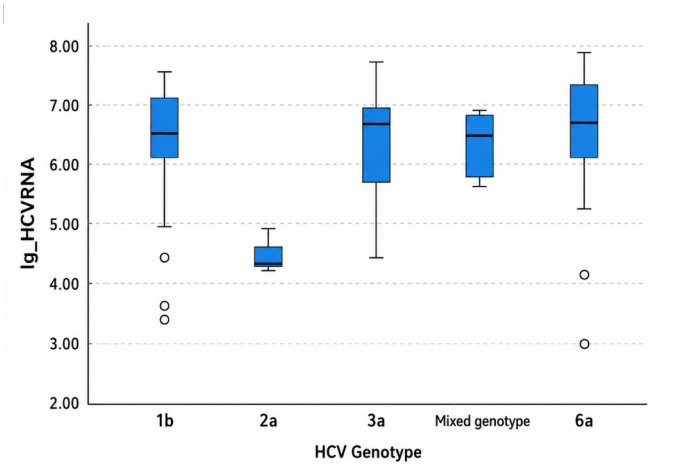

Figure 3 | Comparison of viral load of different HCV genotypes

Table 4 | Comparison of liver function test results for different HCV genotypes [M(P25~P75)]

index	1b	2a	3a	6a	Hybrid	H value	p -value
ALT(U/L) (n=115)	47.80 (25.20~96.20)	27.80 (16.00~27.80)	79.60 (47.83~138.23)	84.70 (13.85~139.05)	37.60 (18.30~72.40)	7.159	0.128
AST(U/L) (n=115)	59.00 (29.25~93.55)	59.30 (31.60~59.30)	62.35 (43.93~136.93)	61.00 (42.60~106.08)	43.00 (24.50~72.20)	6.29	0.178
ALP(U/L) (n=102)	32.60 (21.05~76.15)	46.80 (19.30~46.80)	80.50 (29.90~132.95)	70.60 (41.55~187.70)	56.40 (17.20~143.05)	5.824	0.213
GGT(U/L) (n=100)	76.10 (51.40~95.45)	88.20 (62.00~88.20)	109.50 (66.55~155.65)	128.40 (55.00~168.30)	77.80 (58.15~104.50)	6.149	0.188
TBIL (μmol/L) (n=115)	18.10 (12.15~23.50)	24.70 (23.40~24.70)	17.95 (13.48~31.43)	13.80 (7.53~19.60)	13.10 (10.10~20.30)	8.324	0.8
DBIL (μmol/L) (n=114)	4.50 (3.00~6.95)	6.60 (6.50~6.60)	4.50 (3.45~8.90)	4.15 (2.10~7.33)	2.85 (2.28~4.33)	12.636	0.013
TBA (μmol/L) (n=113)	8.00 (3.45~14.40)	25.00 (25.00~25.00)	9.10 (4.35~51.70)	6.75 (4.85~11.30)	5.70 (2.68~10.83)	3.955	0.412

Table 5 | Comparison of liver fibrosis detection results for different HCV genotypes [M(P25~P75)]

Index	1b (n=31)	2a (n=3)	3a (n=7)	6a (n=15)	Mixed type (n=2)	H value	p -value
HA (ng/mL)	128.00 (72.90~190.00)	158.00 (76.70~158.00)	100.00 (57.10~140.00)	93.30 (76.00~139.00)	194.05 (58.10~194.05)	2.39	0.664
CIV (ng/mL)	26.60 (18.10~51.60)	50.90 (36.80~50.90)	24.10 (17.60~56.40)	26.80 (13.90~85.90)	37.00 (21.50~37.00)	2.789	0.594
PIIIP (ng/mL)	33.00 (20.20~50.50)	52.60 (36.10~52.60)	24.90 (10.80~47.70)	39.90 (16.40~83.30)	295.30 (84.60~295.30)	5.243	0.263
LN (ng/mL)	68.10 (40.60~113.00)	561.00 (122.00~561.00)	51.60 (30.30~114.00)	96.30 (53.80~220.00)	65.60 (31.20~65.60)	8.367	0.079

Correlation analysis between HCV genotype and liver function indicators

Liver function biochemical indicators are important indicators for assessing the degree of liver damage in patients with chronic hepatitis C. Different HCV genotypes may differ in viral replication, pathogenicity, and the degree of liver damage induced due to differences in their biological characteristics. Exploring the relationship between HCV genotype and liver function indicators is of great significance for clinical assessment and prognosis. This study compared and analyzed the levels of ALT, AST, ALP, GGT, TBIL, DBIL, and TBA in patients with different HCV genotypes. The results showed no statistically significant differences in ALT, AST, ALP, GGT, TBIL, and TBA levels among different genotypes ($P > 0.05$). Only the DBIL level showed a statistically significant difference among different genotypes ($H = 12.636$, $P = 0.013 < 0.05$). Post-hoc multiple comparison analysis did not find pairwise differences between specific genotypes, suggesting that this difference may be due to uneven distribution within groups. See Table 4.

Correlation analysis between HCV genotype and liver fibrosis markers

Liver fibrosis is a key pathological stage in the progression of chronic hepatitis C to cirrhosis. Serological liver fibrosis markers (HA, CIV, PIIIP, LN) directly reflect the dynamics of extracellular matrix synthesis and degradation, and are important biomarkers for assessing the degree of liver fibrosis, predicting disease progression, and prognosis. Different HCV genotypes exhibit significant differences in viral replication efficiency, host immune response induction, and liver tissue inflammation patterns, affecting the occurrence and progression rate of fibrosis. Exploring the relationship between HCV genotype and liver fibrosis markers is of great significance for clinical assessment and prognosis. This study compared and analyzed the levels of HA, CIV, PIIIP, and LN in patients with different HCV genotypes. The results showed no statistically significant differences in liver fibrosis markers (HA, CIV, PIIIP, LN) among different genotypes ($P>0.05$). Post-hoc multiple comparisons did not reveal pairwise differences between specific genotypes, which may be related to the small sample size of some genotypes and uneven distribution among groups. See Table 5.

Detection performance analysis

The main evaluation indicators were: the positive concordance rate of the experimental group was 100.00% (95% CI: 94.50%~100.00%), and the negative concordance rate was 100.00% (95% CI: 96.79%~100.00%), which was completely consistent with the test results of the control group.

Secondary evaluation indicators: Overall concordance rate 100.00% (95% CI: 97.93%–100.00%); Kappa coefficient 1.0000 (95% CI: 1.0000–1.0000), indicating that the two detection methods are almost identical. The concordance rates for each subtype were 100.00%, with positive concordance rates of 95% CI for type 1b (90.11%–100.00%), 72.25%–100.00% for type 3a (95% CI: 72.25%–100.00%), 43.85%–100.00% for type 3b (95% CI : 43.85%–100.00%), and 85.13%–100.00% for type 6a.

Data quality analysis

Overall data indicators: The effective sequencing throughput of the experimental instruments ranged from 187.95 to 372.9 M reads, all of which were no less than 100 M reads; the base recognition quality index (Q30) ranged from 82.65% to 92.63%, all of which were no less than 80%. The average raw data volume (number of bases) of the total sample was 141,307,9978.02, the average ratio of high-quality data to overall coarse data was 71.11%, the overall target region coverage was 99.98%, and the overall target region uniformity was 1.00. All indicators met the expected requirements.

Sample quality control indicators: Sequencing data volume (Raw reads) 1,922,416~11,130,218; total number of sequencing bases (Raw bases) 576,724,800~333,906,5400;

Q30 base percentage 86.46%~97.63%; sequence alignment to reference genome percentage 97.02%~99.98%; target region coverage 97.03%~100.00%; average sequencing depth 492,507.90~5,777,965.46, all meeting the quality control targets.

Stratified analysis: After stratifying the total number of sequencing bases, there were no significant differences in the effective sample base recognition quality value Q30, the proportion of sequence aligned to the reference genome, and the target region coverage among the different regions (P=0.2868, 0.2422, 0.7553, respectively); there were no significant differences in the positive judgment value (RPM) of effective positive and negative samples among the different regions (P>0.05 for all), and the detection performance of each subgroup was consistent.

Safety evaluation

Five batches of the test equipment were run without any malfunctions. The equipment stability, safety, and ease of use were all rated at 100%. No system-wide leakage, loose or detached components, or abnormal software interruptions occurred. The researchers were satisfied with all six evaluation criteria , including ease of operation and software usability. No adverse events, serious adverse events, or equipment defects occurred throughout the entire testing process.

Discussion

Hepatitis C is a disease caused by HCV infection, characterized by liver damage. It is one of the main causes of serious liver diseases such as cirrhosis and liver cancer [1]. HCV is a single positive-sense RNA virus. Due to the lack of proofreading activity of HCV NS5B RdRP , the fidelity is low, resulting in a high error rate during replication, which promotes the genetic heterogeneity and population complexity of HCV. It has now been divided into at least 7 genotypes and multiple subtypes . The distribution of HCV genotypes has obvious geographical differences. Understanding the genotype prevalence characteristics in specific regions is the basis for improving the prevention and control strategy of hepatitis C [8]. In northern China, types 1b and 2a are the main types, while types 3 and 6 are more prevalent in southern China [9]. As time goes by, population mobility intensifies and transmission routes diversify, the epidemiological patterns of HCV genotypes in various regions also change. HCV genotyping is not only a key basis for guiding the selection of direct antiviral drugs (DAA), but also an important basis for assessing disease progression, judging prognosis and carrying out regional prevention and control [2]. Therefore, understanding the distribution characteristics of HCV genotypes in this region and clarifying the HCV genotyping of hepatitis C patients is of great clinical significance for diagnosis and treatment. This study is the first to use next-generation sequencing (NGS) technology to perform genotyping on 133 anti - HCV positive patients in Yueyang area and explore its correlation with gender, age, viral load, liver function and liver fibrosis indicators.

This study shows that the most common HCV genotype in Yueyang is type 1b (42.9%), followed by type 6a (26.3%), type 3a (10.5%), mixed type (4.5%), and type 2a (2.3%). The results are consistent with the survey on hepatitis C virus infection in Yueyang by Liu Feng et al. [10], showing obvious regional characteristics in central China. Multicenter epidemiological data across the country show that type 1b is the absolute dominant subtype in mainland China, accounting for 52.18%~62.78%, followed by type 2a; type 6a was previously more common in South China and Southwest China, but has shown a significant upward trend in Central China in recent years [11]. In this study, the proportion of type 6a reached 26.3%, which is significantly higher than the national average. From the perspective of regional epidemiology, Yueyang is located in the northeast of Hunan Province, and the Beijing-Guangzhou Railway runs through the area. It is an important regional transportation hub and a key node connecting Central China and South China. Its HCV genotype distribution retains the characteristics of type 1b as the traditional dominant strain in the central region of China. At the same time, the proportion of type 6a has increased significantly, approaching the prevalence level in South China. This suggests that Yueyang is a transitional zone between the prevalence of HCV in Central China and South China. This unique distribution pattern is closely related to geographical location, population flow and transmission chain, and has important regional representativeness. The proportion of type 2a is relatively low, which is consistent with the gradual decline in the spread of type 2a throughout the country [9]. Mixed infection accounted for 4.5%, suggesting the possibility of repeated exposure or multiple infection. Previous domestic studies have mostly used fluorescent PCR or reverse dot hybridization technology to detect HCV genotypes. This study used the NGS high-throughput system for detection, which has higher accuracy and more reliable type identification, and is superior to traditional typing methods. The above-mentioned HCV genotype distribution characteristics are of practical significance for hepatitis C prevention and control in Yueyang. The high proportion of genotype 1b indicates that existing infections related to previous blood transmission remain an important component; the high prevalence of genotype 6a suggests the existence of active transmission chains in the region, requiring strengthened management of the source of infection and control of transmission routes. Furthermore, clarifying the local HCV genotype distribution helps clinicians directly select targeted antiviral treatment regimens, improving treatment success rates.

HCV RNA viral load directly reflects the degree of viral replication activity and is a core laboratory indicator for judging disease progression and monitoring the efficacy of DAA treatment [12]. The results of this study showed that the median viral load of HCV 1b, 6a, 3a and mixed HCV infections were all at a high level, and there was no statistically significant difference between the groups (P=0.054 > 0.05).

Multiple domestic and foreign studies have confirmed that there is no clear correlation between HCV genotype and viral load [13, 14]. The level of viral replication is mainly affected by the host's immune status, duration of infection, and complications, rather than the genotype itself. In this study, 66.2% of patients had high viral load, suggesting that the overall viral replication of HCV infected individuals in this region is relatively active, but this characteristic is not significantly related to genotype.

Serum ALT, AST, ALP, GGT, TBIL, DBIL, TBA, and other liver function indicators can directly reflect the degree of hepatocellular damage and the excretory and metabolic function of the hepatobiliary system. This study showed no statistically significant differences in ALT, AST, ALP, GGT, TBIL, and TBA among different HCV genotypes, except for DBIL (H=12.636, P=0.013). However, subsequent multiple comparisons did not reveal clear differences between genotypes, suggesting that this result may be due to fluctuations in group distribution and lacks clear clinical interpretability. Multiple clinical observations in Wuhan, Jiangsu, Qinghai, and other regions in China have confirmed that there is no stable association between HCV genotype and liver function indicators [14, 15]. Elevated transaminases mainly reflect host immune- mediated liver inflammation rather than the direct pathogenicity of the viral genotype itself. Although some reports suggest that type 3 liver injury is more significant, these are often due to selection bias or confounding factors such as alcohol consumption and fatty liver, and large-scale unbiased cohort studies have mostly shown negative results [16, 17]. Our results further support that the degree of liver function abnormalities in hepatitis C patients mainly depends on host-related factors rather than HCV genotype differences.

Liver fibrosis is the core pathological link in the progression of chronic hepatitis C to cirrhosis. Serum HA, CIV, PIIIP, and LN liver fibrosis indicators can reflect the dynamics of extracellular matrix synthesis and degradation, and are classic and commonly used indicators for non-invasive clinical assessment of liver fibrosis [18]. This study showed that there were no statistically significant differences in the four liver fibrosis indicators among different HCV genotypes (P>0.05), which is consistent with the consensus of experts on non-invasive laboratory diagnosis of liver fibrosis and the conclusions of many clinical studies at home and abroad [14, 19]. The progression of liver fibrosis is the result of the combined effects of long-term chronic injury, inflammation repair and host metabolism. It is affected by multiple factors such as age, unhealthy drinking, obesity, and diabetes, and has no independent strong correlation with HCV genotype [20]. Viral load and genotype are not independent predictors of liver fibrosis progression, and fluctuations in serological markers are difficult to reflect the differences between genotypes [21, 22]. These results suggest that when assessing liver fibrosis in patients with chronic hepatitis C, a comprehensive consideration of liver fibrosis risk factors should be taken into account, and judgment should not be based solely on HCV genotype.

The accuracy of detection technology directly affects genotyping and treatment selection. This study used the FASTASeq 300 Dx gene sequencer, based on sequencing-by-synthesis technology, which boasts high throughput and high sensitivity. Results showed that the sequencer achieved a 100.00% positive concordance rate, negative concordance rate, and overall concordance rate, with a Kappa coefficient of 1.0000, completely consistent with traditional PCR-fluorescent probe methods. Furthermore, the concordance rate for each subtype was 100.00%, indicating extremely high accuracy in HCV genotyping. In addition, the sequencing throughput, Q30 base ratio, and other data quality indicators met quality control requirements, and the target region coverage reached 99.98%, ensuring the reliability of the genotyping results. Regarding safety, no adverse events or instrument defects occurred during the experiment. The equipment stability, safety, and ease of use were all rated at 100.0%, demonstrating the sequencer's good safety and practicality in clinical applications and its suitability for routine HCV genotyping. Compared with traditional Sanger sequencing, next-generation sequencing technology can detect multiple genotypes at the same time without the need to design specific primers for different subtypes, which greatly improves the detection efficiency and is especially suitable for large-scale screening in areas with multiple subtypes [23].

This study also has certain limitations. As it is a single-center study, it only included patients treated at our hospital, which introduces selection bias and limits representativeness. Due to geographical factors, the sample size for HCV 2a and mixed subtypes is limited, resulting in limited statistical power. Confounding factors such as prior antiviral treatment, hepatitis B co-infection, alcohol consumption, and metabolic diseases were not adjusted for; only univariate analysis was performed, without multivariate regression, and no follow-up analysis was conducted on patients' clinical outcomes and treatment responses. Future research should expand the sample size to include patients from different levels of medical institutions in Yueyang, and incorporate more laboratory serological indicators and confounding factors for multivariate analysis. Long-term follow-up of treatment responses and disease progression in patients with different genotypes is necessary to provide more reliable scientific evidence for further in-depth research into the immunopathological mechanisms of HCV infection and for precise prevention and control of hepatitis C and individualized diagnosis and treatment of HCV infection in Yueyang.

Conclusion

In Yueyang District, the predominant HCV genotype was 1b, followed by 6a, 3a, mixed, and 2a. There were no statistically significant differences in viral load, sex, age, ALT, AST, ALP, GGT, TBIL, TBA, HA, CIV, PIIIP, and LN among different genotypes, but a statistically significant difference was observed in DBIL. The FASTA Seq 300 Dx gene sequencer demonstrated extremely high accuracy and safety in HCV

genotyping, providing reliable genotypic evidence for individualized clinical treatment of hepatitis C.

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