



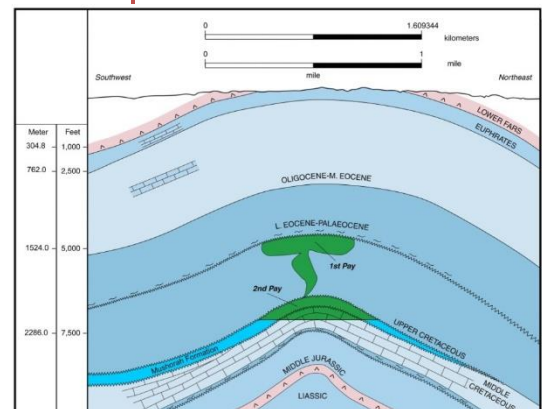
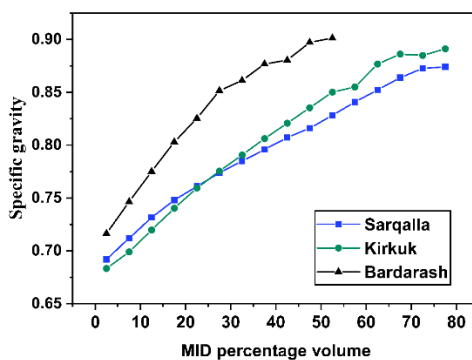
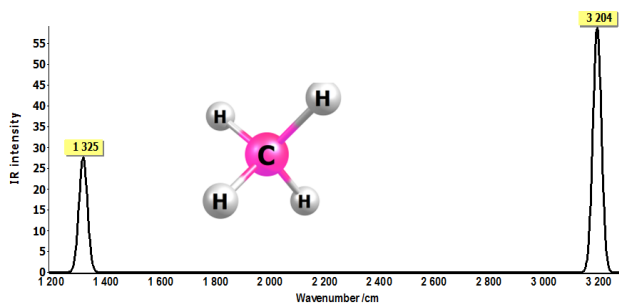
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## Seroprevalence and Genotypic Distribution Patterns of Hepatitis C Virus among Infected Patients from Sulaimaniyah Province: Iraq

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### Abstract

**Background:** The hepatitis C virus (HCV) is a prevalent cause of liver illness. The virus displays a high degree of genetic variability, with documented geographical differences in genotype predominance. **Objective:** This study aimed to determine the prevalence of HCV genotypes among people who lived in Sulaimaniyah province, Kurdistan region of Iraq. **Patients and Methods:** Blood samples were collected from 229 individuals identified as having a confirmed positive HCV antibody and who had been referred to the Public Health Laboratory in Sulaimaniyah city by medical professionals for genotyping from July 2021 to December 2022. Following the extraction of the viral RNA, a PCR-based genotyping kit was used to identify the HCV genotype. **Results:** The most prevalent genotype was GT1a (31.6%), followed by GT1 and GT4 (23.6%) and then GT1b (7.9%). Mixed genotypes were observed in 10.4% of samples. Males were affected mainly by HCV (58.5%) than females (41.5%). The frequency of GT1a was higher in males (32.1%) compared to females (30.5%). On the other hand, males exhibited a more excellent distribution of GT4 and GT1 than females, while GT1b and GT5 were observed more in females than males. Among mixed genotypes, GT5 or 6 was the most prevalent infection type (no.=8, 3.5%), while GT1a, 3 reported the lowest rate (no.=1, 0.4%). **Conclusion:** This study assesses the HCV genotype distribution among infected HCV patients in Sulaimaniyah, which is distinct from the prevalent distribution in Iraq and Middle Eastern Arab nations, but it is equivalent to the distribution worldwide.

### Introduction

Acute hepatitis C virus (HCV) infection becomes chronic in most cases (55–85%). Chronic disease can either lead to mild illness or develop into liver cirrhosis, liver failure and hepatocellular carcinoma (HCC). Out of 100 people infected with HCV, around 60–70 develop chronic liver disease, 5-20 build cirrhosis between 20-30 years, and 1-5 die from the consequences of cirrhosis or HCC (1).

HCV is a member of the family Flaviviridae, genus Hepacivirus, and the subgenus Hepacivirus (2). The genome is 9.6 kb and encodes a polypeptide of about 3000 amino acids in a single open reading frame. To a large extent, its positive RNA displays genetic diversity (3,4).

HCV is a dangerous blood-borne virus that affects people all over the world. Around 71 million individuals are chronically infected with HCV worldwide, putting pressure on healthcare systems everywhere. With an estimated 15 million people living with chronic HCV infection, the Middle East and North Africa are the most hit by HCV infection globally (5).

HCV infections are currently divided into eight different genotypes (GT1-GT8) and have 90 known subtypes. Around 54.3 million cases (30.1%) of the second most prevalent genotype (GT3) in the worldwide population. The number of people with this genotype is highest in South and Central Asia, but there are many people with this genotype all over the world who use drugs (6).

GT8, a single phylogenetic group from previously known sequences, was found in four Punjabi patients who were not epidemiologically related. HCV GT1–3 circulates widely, while 46% of HCV cases are GT1. In Japan, 73% of identified HCV infections are subtype 1b. In North America, Europe, and Australia, subtypes 1a and 1b are the most frequent. HCV GT4 is most frequent in Africa and the Middle East, while GT 5 and 6 are limited to Southern Africa and Southeast Asia, respectively. GT 1, 2, 3, 4, and 6 have many subtypes and genetic variations. Until now, GT5 and the newly identified GT 8 had a single subtype (7).

There is a significant effect on the therapy, duration, response rate, and efficacy of current and novel antiviral treatments based on the HCV genotypes determined before treatment (7). Thus, we planned to assess the prevalence of HCV genotypes in the Sulaimaniyah area of the Kurdistan region of the Republic of Iraq to provide more up-to-date information on the frequency of HCV genotypes available in the area.

## **Materials and Methods**

### *Sample size and study setting*

A total of 229 positive HCV patients at the Teaching Hospital for Gastroenterology and Hepatology in Sulaimaniyah, Iraq, were enrolled in this study from July 2021 to December 2022.

### *Questionnaire*

The patient's sociodemographic characteristics (gender) were taken using a well-designed, self-created questionnaire.

### *Inclusion criteria*

Patients with confirmed HCV were enrolled regardless of age, gender, ethnicity, and nationality.

### *Exclusion criteria*

Patients with other types of hepatitis viral infection rather than HCV were excluded from the study.

### *Ethical consideration*

The scientific and ethics committees of the College of Nursing and the College of Medicine at the University of Sulaimani, Sulaimaniyah, Iraq approved this study. The final administrative preparations (IRB) to obtain an official agreement for the actual data collected by the Sulaimani General Directorate of Health and the Sulaimani General Teaching Hospital, Iraq, were also done. The patients filled out a

written consent form to allow the research to be conducted on their blood samples. Also, they felt free to leave the study without giving any reason. Patient confidentiality also was preserved.

### *Study protocol*

#### *Blood samples*

From July 2021 to November 2022, 229 people (134 men and 95 women) who tested positive for anti-HCV by commercial ELISA kit (LIAISON® XL HCV Ab, Via Crescentino, snc 13040 Saluggia (VC), Italy) were referred by medical experts to the Public Health Laboratories in Sulaimaniyah province for genotyping. Then, about 10 mL of blood sample was obtained from each patient into EDTA tubes. Serums were removed from samples after centrifugation and kept at -70 °C for further use.

#### *RNA extraction and genotyping*

Using the automated EZ1 advanced XL nucleic acid extraction machine (Qiagen, Germany) and the EZ1® Virus Mini Kit v2.0, the HCV genome (RNA) was isolated from 140 µL of serum. This was done following the instructions provided by the manufacturing companies. Complementary DNA (cDNA) was manufactured by following the instructions provided by the manufacturer of the REVERTA-L Reverse Transcription reagent kit (AmpliSens, Russia). Then, 10 µl of extracted HCV-RNA were incubated in the GeneAmp PCR system 9700 (Applied Biosystems) at 37 °C for 30 minutes along with 5 µl of RT-G-mix-1 and 6 µl of murine leukaemia virus reverse transcriptase. A commercial DNA extraction reagent kit (MagPurix® ZP02006, China) was used to discriminate and qualitatively detect HCV genotypes using real-time polymerase chain reaction (RT-PCR) with hybridization fluorescence detection according to the manufacturer's instructions. For amplification and the detection of a specific area in the pathogen's genome, particular primers and fluorescent dyes connected to oligonucleotide probes were used. During the process of RT-PCR, the amplified products were identified by their specific binding to oligonucleotide probes, including 1, 1a, 1d, 2, 3, 4, 5 or 6; and a mixed number of 1, 2; 1, 3; 1, 4; 1a, 3; 1b, 4; and 3, 4.

#### *Anti-HCV antibody detection*

It usually takes a few weeks for the immune system to produce enough antibodies to be identified by an antibody test, but it might take up to six months. Therefore, the enzyme-linked immunosorbent assay (ELISA) and the recombinant immunoblot assay were used to detect HCV antibodies (RIBA). First, an ELISA kit of the fourth generation (ELISA LIAISON® XL HCV Ab, Via Crescentino, snc 13040 Saluggia (VC) Italy) was used in conjunction with a fully automated microtiter plate analyzer (ETI-MAX 3000 system, STRATEC Biomedical Systems AG, Italy) to test serum samples for the presence of anti-HCV antibodies. In a nutshell, serum from patients was mixed with recombinant HCV-specific antigen and incubated in 96-well plates that had previously been coated. After that, the sample and any antibodies that were not needed were removed by washing. Following that, other conjugates were included. After the incubation period, the plates were cleaned. Then tetramethylbenzidine (TMB) and hydrogen peroxide (substrate solution) was added for detection after adding sulphuric acid, which halted the process. Finally, a third-generation immunoblot assay (MP Diagnostics HCV BLOT 3.0 kit, MP Biomedicals, Singapore) was utilized to confirm the presence of positive anti-HCV reactivity. This assay featured six distinct bands containing a unique antigen derived from the core, NS3-1, NS3-2, NS4, and NS5 proteins. According to the instructions provided by the manufacturer, samples were

considered negative if they did not react to any of the bands, indeterminate if they only reacted to one round, and positive if they responded to at least two bands.

### Statistical analysis

The collected data were coded in Microsoft Excel and then exported to SPSS (version 25.0) software. Descriptive statistical methods such as the t-test and chi-square were used.

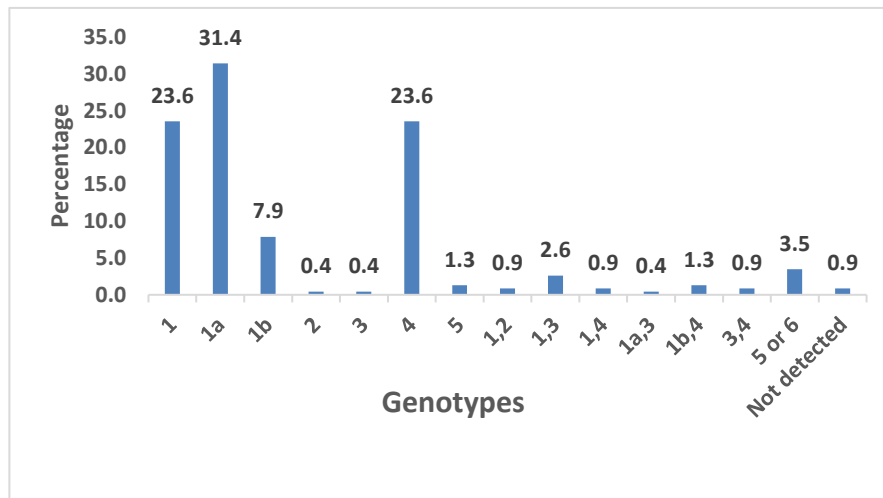
### Results

Figure 1 presents the genotype distribution among 229 HCV-positive patients. Single genotypes, including GT1, GT2, GT3, GT4, and GT5, in addition to two sub-type of GT1 (GT1a and GT1b) were detected. Moreover, mixed genotypes (also known as "more than one type"), including GT1,2, GT1,3, GT1,4, GT1a,3, GT1b,4, GT3,4, and GT5 or 6, were identified among tested patients. Single serotypes (no.=203, 88.6%) were more highly reported than mixed genotypes (no.=24; 10.5%). Both GT1 and GT4 were the major genotypes (no.=54; 23.6%), and GT1a was found to be the HCV subgenotype with the highest incidence (no.=72; 31.4%), while GT2, GT3, and GT1a,3 had lowest frequencies among genotypes (0.45%). Additionally, HCV genotypes were not detected in 2 patients (0.9%) (Table 1).

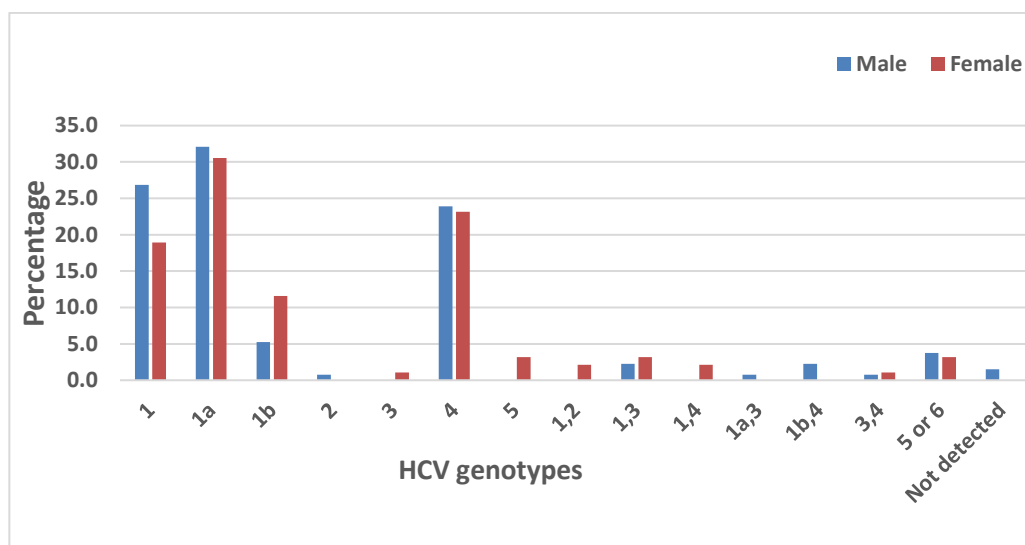
On the other hand, the HCV genotype distribution in the patients was also established according to gender. The rate of HCV infection in males was greater (no.=134, 58.5%) than in females (no.=95, 41.5%). In addition, males exhibited a greater distribution of GT1, GT2, GT4, GT1a, 3, GT1b, 4, and GT5 or 6 than females, while GT1b, GT3, GT5, GT1,2, and GT1,4 were observed more in females than males (Figure 2).

**Table 1.** Frequency distributions of HCV genotypes among studied patients.

| Detected genotype      | Total       |             | Male        |              | Female      |              |
|------------------------|-------------|-------------|-------------|--------------|-------------|--------------|
|                        | (Number, %) | (Number, %) | (Number, %) | (Number, %)  | (Number, %) | (Number, %)  |
| <b>Single genotype</b> |             |             |             |              |             |              |
| 1                      | 54          | 23.6        | 36          | 26.9         | 18          | 18.9         |
| 1a                     | 72          | 31.4        | 43          | 32.1         | 29          | 30.5         |
| 1b                     | 18          | 7.9         | 7.0         | 5.2          | 11          | 11.6         |
| 2                      | 1.0         | 0.4         | 1.0         | 0.7          | 0.0         | 0.0          |
| 3                      | 1.0         | 0.4         | 0.0         | 0.0          | 1.0         | 1.1          |
| 4                      | 54          | 23.6        | 32          | 23.9         | 22          | 23.2         |
| 5                      | 3.0         | 1.3         | 0.0         | 0.0          | 3.0         | 3.2          |
| <b>Total</b>           | <b>203</b>  | <b>88.6</b> | <b>119</b>  | <b>58.6</b>  | <b>84</b>   | <b>41.4</b>  |
| <b>Mixed genotypes</b> |             |             |             |              |             |              |
| 1,2                    | 2.0         | 0.9         | 0.0         | 0.0          | 2.0         | 2.1          |
| 1,3                    | 6.0         | 2.6         | 3.0         | 2.2          | 3.0         | 3.2          |
| 1,4                    | 2.0         | 0.9         | 0.0         | 0.0          | 2.0         | 2.1          |
| 1a,3                   | 1.0         | 0.4         | 1.0         | 0.7          | 0.0         | 0.0          |
| 1b,4                   | 3.0         | 1.3         | 3.0         | 2.2          | 0.0         | 0.0          |
| 3,4                    | 2.0         | 0.9         | 1.0         | 0.7          | 1.0         | 1.1          |
| 5 or 6                 | 8.0         | 3.5         | 5.0         | 3.7          | 3.0         | 3.2          |
| <b>Total</b>           | <b>24</b>   | <b>10.5</b> | <b>13</b>   | <b>54.16</b> | <b>11</b>   | <b>45.83</b> |
| <b>Not detected</b>    | 2.0         | 0.9         | 2.0         | 1.5          | 0.0         | 0.0          |
| <b>Total</b>           | <b>229</b>  | <b>100</b>  | <b>134</b>  | <b>58.5</b>  | <b>95</b>   | <b>41.5</b>  |



**Figure 1.** Frequency distribution of HCV genotypes in studied patients.



**Figure 2.** Distribution of HCV genotypes among the studied patients according to gender.

## Discussion

Hepatitis C viral infection spreads worldwide, infecting 71 million individuals. 75–85% of hepatitis C patients will develop chronic diseases. Chronic HCV infection steadily damages the liver, increasing the risk of cirrhosis, hepatocellular cancer, liver failure, and death (8). HCV is an RNA virus having eight recognized genotypes and 86 identified subtypes that vary by at least 15–25% (9). Direct-acting antiviral, a novel HCV treatment, works well except for specific genotypes. Genotype distribution has changed significantly following the transmission route shift. Thus, understanding HCV genotype distribution improves HCV infection management (10). In this study, GT1a (31.4%) was the most common HCV genotype, followed by GT1 and GT4 (23.6%), GT1b (7.9%), GT5 or 6 (3.5%), GT5 and GT1b,4 (1.3%), GT1,2 and GT1,4 (0.9%), and GT2, GT3, GT1a,3 (0.4%).

These findings match worldwide HCV genotype estimates, in which GT1, GT2, and GT3 are found more frequently. Globally, 46% of HCV patients have GT1. In Japan, 73% of HCV-infected people have subtype 1b. In North America, Europe, and Australia, subtypes 1a and 1b are predominant. GT3 is the second most common (30%) genotype globally, mostly found in South Asia, and is overrepresented among persons who inject drugs worldwide. GT4 is most common in Africa and the Middle East, whereas Gt5 and 6 are found in Southern Africa and Southeast Asia, respectively. Multiple subtypes make up GT1, GT2, GT3, GT4, and GT6. HCV GT7a was found in a Congolese patient in 2006, and GT7b was found in another patient from the same location. However, GT5 and GT8 have one subtype apiece (11).

Previous Iraqi reports and the present work's statistics are comparable. HCV GT4 was the most frequent among Iraqi chronic hepatitis patients (46.68%), followed by GT1 (37.12%) in a six-year cross-sectional study. In 2017, GT1 (52.63%) dominated HCV genotype distribution. In Erbil and Basra, GT1a, GT1b, GT2, and GT3a were 37.25%, 17.65%, 4.90%, and 0.98%, respectively. These investigations also found mixed genotypes (14.7%) (12-14).

HCV GT4 predominates in Iraq and other Middle Eastern nations. India, Nepal, and Pakistan have considerable expatriate communities in Bahrain, Dubai, and Oman, where GT1 and GT3 are most frequent. GT1 and GT3 had the greatest rates. Thailand, Malaysia, India, Pakistan, and Mainland China also have HCV GT3 infections. Only one GT5a case was found in Punjab, Syria, whereas GT5 and GT6 were found in South Africa and Southeast Asia [13]. Several nations in the Middle East, including Saudi Arabia (48.3%), Egypt (85%), and Lebanon (45.7%), have a GT4 population that is predominant (15). On the other hand, the HCV GT1 strain is the one that is found in the highest numbers in non-Arab nations in the area, such as Turkey, Iran, Cyprus, and Israel (16).

In 219, a study in Duhok province realized that HCV positives were 50.0% (30/60), including GT4 at 43.3% (26/60) and GT3 at 6.7% (4/60). The G5 genotype was discovered at 1.3% frequency. Two prior Iraqi investigations showed no genotype in any instance. Therefore, our 0.9% prevalence of mixed GT 3,4 was not unexpected and has been observed in prior Iraqi experiments. However, the authors have established that GT2 is not present among Baghdad dialysis patients in previous studies, while indefinite or mixed genotypes caused 3% of all HCV infections. Infection with a specific HCV genotype does not protect against disease with other HCV genotypes (12, 13,17).

HCV genotype gender distribution was also examined in this study, and it was found that males and females have different HCV genotype distribution ratios. GT3, GT1b, GT5, and mixed GT1,2; GT1,3, G1b, 4 predominated in females, whereas GT 1,1a, 2,4, and mixed 1a, 3; 1b, 4; 3,4 predominated in men. Another research found that females were more likely to have GT1b than males (18). However, in Turkey, HCV genotype distribution did not vary by gender (19).

The type of test, sample size, and patient type may explain the differences in genetic patterns between this research and others from Iraq (hemodialysis and thalassemia and asymptomatic). This discrepancy in genotype patterns and recording of GT1a, 5 and mixed G3,4 in the current study suggests that after the major political changes in Kurdistan over the last two decades, the country was exposed to increased air travel and international exchange, which could introduce new microorganisms or strains. In addition, travelling, immigration, outside treated patients, matrimonies, kinfolk, ethnic, and population migrations in our region may spread these HCV genotypes from European or Asian nations, including Iran and Turkey. However, further research is needed to corroborate these findings. The present study has some drawbacks, including a need for clinical data to determine if clinical variables affected HCV

genotype distribution. In addition, the study population might reflect something other than the overall population.

## Conclusions

The most common HCV genotype in Sulaimaniyah Province was 1a (31.6%), followed by 1 and 4, which had the same percentage (23.6%), and 1b (7.9%). Mixed genotypes were observed in 10.4% of the samples needed to determine better the possible role of genotype in the outcome of HCV-related liver disease.

## Funding

The authors have received no funding for the research.

## Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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