ISSN: 2635 - 2826

Journal of Clinical & Biomedical Research

SCIENTIFIC Research and Community

Research Article

Open Access

Prevalence of Mixed Infections in HCV Chronically Infected Patients

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ABSTRACT

Introduction: Mixed infections by different HCV genotypes constitute an important concern worldwide. The aim of this study is to determine the prevalence of mixed infections by two or more HCV genotypes in HCV chronically infected patients.

Materials and Methods: 100 HCV-RNA positive patients were included in this study. This retrospective study was conducted according to the Helsinki Declaration. Informed consent of patients was not compulsory due to its retrospectivity. HCV-RNA was detected by means of Cobas Taqman 48 with High Pure System, Roche. Sequencing was performed by Trugene® HCV 5'NC Genotyping Kit (Siemens). Statistics were performed with SPSS version 25.0.

Results: mixed infections were detected in 15 patients (15.0 %, 8 male and 7 female). Mixed infections were as follows: 3 patients were infected by types 1a+1b, 3 by types 2a+2c, 2 by types 3a+3c, 1 by types 3a+3d, 1 by types 3a+3d+3e, 2 by types 3a+3e, 2 by 4a+4d and 1 by types 4a+4c+4d. Mixed infections were equally distributed among male and female patients (p-value = 0.402). The mean age of individuals infected only with one HCV type was 41.9 years old; while of those mix-infected were 51.9. Statistically significant differences of mix infections distribution according to age were detected (p-value = 0.032). There were no cases with mixed infections across types.

Conclusions: Mixed HCV infections have been reported in many studies. Their prevalence varies worldwide and regional differences exist in their distribution. Mixed infection prevalence depends on the risk factors in the population under study. In this study, the prevalence of mixed HCV infections was 15.0 % and all patients belonged to high-risk groups. Moreover, all mix-infected patients had long-lasting disease course and liver cirrhosis.

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Received: February 06, 2026; **Accepted:** February 12, 2026; **Published:** February 21, 2026

Keywords: Hepatitis C Virus, Genotype, Phylogenetic Tree, Sequences, Mixed Infections, Subtype

Introduction

Hepatitis C virus (HCV) is an enveloped, single-stranded RNA virus belonging to the Flaviviridae family and represents a major cause of chronic liver disease worldwide. Chronic HCV infection remains a leading driver of progressive hepatic fibrosis, cirrhosis, and hepatocellular carcinoma, contributing substantially to global morbidity and mortality [1].

Transmission of HCV occurs primarily through exposure to infected blood, particularly among people who inject drugs, through unsafe medical procedures, and less frequently through sexual or vertical transmission. The epidemiological burden of HCV differs markedly between geographic regions and population risk groups, reflecting variations in healthcare practices and behavioral factors [2,3].

In Europe, the overall prevalence of chronic HCV infection has been estimated at approximately 1-2%, although considerable heterogeneity exists between countries, with genotype distribution varying across regions. Mediterranean countries, including Greece, demonstrate distinct genotype patterns shaped by historical and migratory factors [4-10].

The introduction of direct-acting antivirals has revolutionized HCV treatment, achieving sustained virological response rates exceeding 95%. Nevertheless, accurate genotyping remains clinically relevant in several settings, particularly in epidemiological surveillance, retreatment decisions, and detection of complex infection patterns such as mixed-genotype infections [11].

HCV is classified into genotypes and subtypes based on phylogenetic analysis of viral sequences. A consensus proposal expanded HCV classification into seven confirmed genotypes and more than 60 subtypes, providing standardized criteria for genotype assignment and nomenclature. Globally, genotype 1 remains the most prevalent, followed by genotypes 3, 2, and 4, with regional differences across Europe, Asia, and Africa.

Mixed infections with more than one HCV genotype represent an important clinical and epidemiological concern. Their prevalence varies widely, from low rates in general populations to higher proportions in high-risk groups, such as individuals with repeated exposures or long-standing infection [12,13]. Mixed infections may influence treatment response, complicate resistance patterns, and require optimized patient management.

Detection of mixed-genotype infections depends strongly on the sensitivity of the genotyping methodology. Commercial assays may fail to identify minor viral populations, and next-generation sequencing has been proposed as a confirmatory tool in ambiguous cases. Sequencing-based genotyping approaches, such as the TRUGENE® HCV 5′NC system, provide reliable subtype discrimination and remain widely applied in routine clinical practice.

Given the clinical importance of identifying mixed infections, further data are needed regarding their prevalence and distribution in chronically infected patients, particularly within high-risk populations.

Aim

The aim of this study was to determine the prevalence of mixed infections involving two or more HCV genotypes among chronically infected HCV-RNA positive patients.

Objectives and Goals

The objectives of the study were: a) to estimate the frequency of mixed-genotype HCV infections in a retrospective cohort, b) to describe the genotype/subtype combinations identified, c) to assess associations between mixed infection status and demographic characteristics, and d) to compare the observed prevalence with published international data.

Methodology

A total of 100 HCV-RNA positive patients were included in this retrospective study. All patients were chronically infected and belonged to high-risk groups.

HCV-RNA was detected using the Cobas TaqMan 48 system with the High Pure System (Roche). Genotyping and subtype identification were performed through sequencing using the TRUGENE® HCV 5′NC Genotyping Kit (Siemens).

Sequencing-based approaches were selected because they provide improved accuracy in subtype assignment compared with some commercial assays, and they allow better recognition of mixed infections when more than one genotype population is present [11].

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0. Categorical variables were compared using appropriate significance testing. A p-value <0.05 was considered statistically significant.

Results

Mixed infections were detected in 15 out of 100 patients, corresponding to a prevalence of 15.0%. The distribution of HCV infections among the patients included in the study is presented in Figure 1. Among the mixed-infected patients, 8 were male and 7 were female. Mixed infections were equally distributed between sexes (p = 0.402). The distribution of mixed infections in the study sample is presented in Figure 2. The mixed genotype combinations detected were:

1a + 1b (3 patients)
2a + 2c (3 patients)
3a + 3c (2 patients)
3a + 3d (1 patient)
3a + 3d + 3e (1 patient)
3a + 3e (2 patients)
4a + 4d (2 patients)
4a + 4c + 4d (1 patient)

The mean age of patients infected with a single genotype was 41.9 years, whereas mixed-infected patients had a mean age of 51.9 years. A statistically significant association between age and mixed infection status was observed (p = 0.032). No cases of mixed infection across different major genotype groups were identified. All mixed-infected patients had long-lasting disease course and liver cirrhosis.

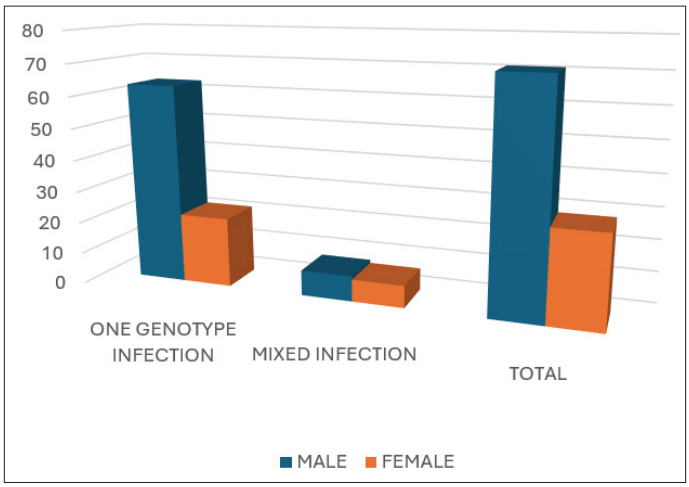

Figure 1: Distribution of HCV Infections Among the Patients Included in the Study

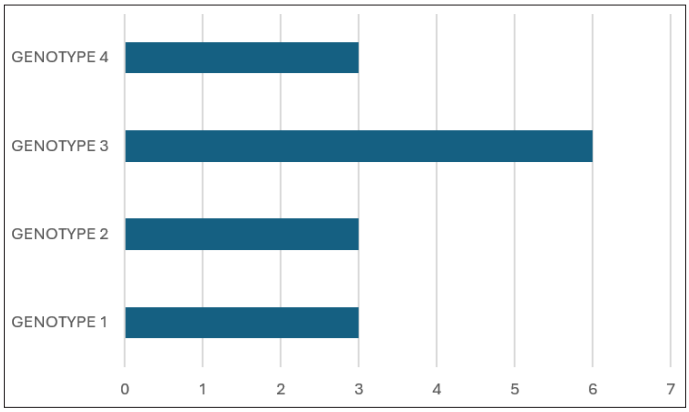

Figure 2: Distribution of Mixed Infections in the Study Sample

Discussion

Mixed HCV genotype infections have been increasingly reported worldwide, with prevalence rates strongly dependent on population risk factors and the sensitivity of genotyping techniques.

In our study, the prevalence of mixed infections was 15.0%, which is higher than that reported in several European studies. Studies identified mixed genotype infections in approximately 10.8% of chronically infected individuals, emphasizing the association with long-term exposure and high-risk transmission routes [3,12,13] .

Other European cohorts have reported substantially lower prevalence. In Turkey, mixed genotype infections were observed in only 1.3% of patients, suggesting important regional variation and differences in study populations, while genotype 4 observed with a low rate [14,15].

The relatively high prevalence detected in our study may be explained by the inclusion of patients from high-risk groups, repeated exposure events, and advanced liver disease. Mixed infections are more likely to occur in individuals with multiple opportunities for reinfection or superinfection over time [16-19].

Accurate classification of HCV genotypes is essential for epidemiological surveillance and clinical management. The expanded classification system proposed by Smith et al. standardized genotype and subtype definitions, allowing consistent global comparisons [20,21].

Our findings included combinations mainly within genotypes 1, 2, 3, and 4, which correspond to the most prevalent genotypes circulating in Europe and the Mediterranean region [1,10].

The detection of mixed infections remains challenging. Fernández-Caso et al. demonstrated that commercial assays may underestimate mixed infections and recommended sequencing or next-generation sequencing confirmation when multiple genotypes are suspected [11]. The use of sequencing-based TRUGENE genotyping in our study provided reliable subtype discrimination, supporting the validity of our results.

Clinically, identifying mixed-genotype infections is important for optimizing patient management, as minor viral populations may emerge under selective antiviral pressure, potentially influencing retreatment strategies [11].

Conclusion

Mixed HCV genotype infections represent a clinically relevant phenomenon with considerable variability in prevalence worldwide.

In this retrospective cohort of chronically infected high-risk patients, mixed infections were detected in 15.0% of cases, with significant association with older age and advanced liver disease. Sequencing-based genotyping remains a valuable approach for detecting mixed infections and ensuring optimal clinical management.

Further large-scale studies are needed to clarify the epidemiological patterns and clinical implications of mixed HCV infections in different geographic regions.

Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki. Informed consent was not required due to the retrospective nature of the study.

Financial Disclosure

The financial support of the study was provided by Maria Kimouli.

Conflict of Interest

The authors declared no conflict of interest.

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