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EPIDEMIOLOGICAL DYNAMICS AND CLINICAL-DEMOGRAPHIC CHARACTERISTICS OF HEPATITIS C IN A POPULATION FROM SOUTHERN ROMANIA: A RETROSPECTIVE STUDY (2021–2024)

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ABSTRACT

Chronic hepatitis C virus (HCV) infection remains a major public health challenge. This retrospective study evaluated the seroprevalence of anti-HCV antibodies in a sample of 5346 patients tested in Alexandria (Teleorman County) between 2021 and 2024 using laboratory enzyme immunoassays. The overall seropositivity rate was 1.53 % (82 cases), with no significant differences between females and males (1,70 % vs. 1,11%, p = 0,1417; OR = 1,54). Age distribution indicated a zero prevalence in the 18–34 age group (0.00 %) and a peak value of 3.70 % in the ≥65 age group (p = 2,75 × 10^{-14}), confirming the persistence of a cohort effect. Temporal dynamics demonstrated a stable annual rate (1.89% in 2021, 1.40 % in 2022, 1.55 % in 2023, and 1.59 % in 2024, p = 0.9262), highlighting the need for continued population screening campaigns and sustained access to DAA therapies.

INTRODUCTION

Hepatitis C virus (HCV) infection remains a major public health challenge, representing a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Turculeanu et al. 2013).

Globally, an estimated 50–58 million people live with chronic infection (WHO 2021; ECDC 2024), compared to historical estimates of 170 million (a prevalence of 3 %) (Sporea & Goldiș 2007).

In Romania, the historical prevalence of 3 %–7 % (Sporea & Goldiș 2007; Gheorghe et al. 2010) has decreased in the direct-acting antiviral (DAA) era to approximately 1.39 %–1.40 % in the general population (Gheorghe et al. 2020).

Hepatitis C virus (*Hepacivirus*, *Flaviviridae* family) (Uțu et al. 2024) causes an infection with a high potential for chronicity and progression to cirrhosis (Stoica & Scripcariu 2016).

The virion is a positive-sense single-stranded RNA virus (50–60 nm), enveloped by a membrane containing glycoproteins E1 (responsible for hepatocyte attachment) and E2, as well as a nucleocapsid enclosing the viral genome (Turculeanu et al. 2013; Botnaru 2006)

Liver involvement operates through a dual mechanism: direct (a cytopathic effect correlated with viremia) and indirect (immune-mediated via autoantibodies and cytotoxic T lymphocytes) (Pântea et al. 2013).

Clinically, the infection is frequently asymptomatic or characterized by asthenia and right upper quadrant discomfort (Sporea & Goldiș 2007).

Through circulating immune complexes, HCV induces numerous extrahepatic manifestations (hematological, renal, endocrine, and dermatological) and is frequently associated with hepatocellular carcinoma (present in 50–75 % of cases) or HBV coinfection (5–10 %) (Pântea et al. 2013; Sporea & Goldiș 2007).

Predictive factors of severity in chronic hepatitis C include age over 40 years at the time of infection, transfusion-associated transmission, male gender, alcohol consumption exceeding 50 g/day, associated metabolic diseases, steatosis, viral coinfections (HBV, HDV, HIV), and humoral immunodeficiencies (Russu et al. 2019).

The link between HCV and hepatocellular carcinoma (HCC) is well established, with over 70 % of HCC patients infected with HCV (Russu et al. 2019).

Transmission routes are predominantly parenteral (transfusions, hemodialysis, nosocomial), sexual (to a lesser extent than HBV), perinatal (in cases of high viral replication or HIV coinfection), and sporadic in over 20 % of cases (Botnaru 2006).

Serological diagnosis relies on enzyme immunoassay screening tests (ELISA/CLIA) for detecting anti-HCV antibodies and confirmatory tests using immunoblotting techniques (RIBA) (Licker et al. 2020).

Functional biochemical tests demonstrate a moderate and fluctuating elevation of ALT, alongside increased levels of IgG and gamma-GTP, whereas liver biopsy remains indicated in seropositive patients with detectable HCV-RNA to assess lesion severity (Botnaru 2006).

In the context of the recommendations of the European Association for the Study of the Liver (EASL 2020) and the WHO goals to eliminate viral hepatitis by 2030, monitoring seroprevalence at the county level is essential.

This study aims to analyze the epidemiological dynamics, anti-HCV antibody seroprevalence, and clinical-demographic characteristics of a population of 5346 individuals investigated at the LABORMED Laboratory in Alexandria (Teleorman County) over a four-year period (2021–2024).

MATERIAL AND METHODS

Study Design and Population Sample: A retrospective, observational study was conducted on a cohort of 5346 patients (3816 females and 1530 males) presenting for routine health evaluations or referred by general practitioners/specialists at the LABORMED Medical Analysis Laboratory in Alexandria, Teleorman County, Romania.

The study evaluated tests performed over a four-year period, spanning from November 1, 2021, to December 31, 2024. Inclusion criteria encompassed all individuals for whom anti-HCV antibody determination was requested and whose demographic data (age, gender) were fully recorded. Duplicate tests performed on the same patient within an interval of less than 30 days were excluded.

Sampling and Temporal Distribution by Year: To monitor the multi-year dynamics of testing and seropositivity rates, the sample was chronologically stratified across the four study years: 2021 (November–December): 264 patients evaluated; 2022: 1644 patients evaluated; 2023: 1807 patients evaluated; 2024: 1631 patients evaluated. This temporal breakdown allowed for a comparison of population addressability rates and the identification of annual variations in anti-HCV antibody seroprevalence.

Laboratory Methodology: Qualitative determination of anti-HCV antibodies in patient serum or plasma was performed using rapid membrane-based immunochromatographic technique and enzyme immunoassay screening methods (ELISA/CLIA) (Licker et al. 2020).

The assay utilizes recombinant HCV antigens immobilized on a nitrocellulose membrane to selectively capture specific anti-HCV antibodies at the test line level, providing high diagnostic sensitivity (>99 %) and specificity (>99.5 %) (ECDC 2023; WHO 2024).

Venous blood samples were collected in anticoagulant-free vacutainers with gel separators, and serum obtained by centrifugation at 3,000 rpm for 10 minutes was analyzed in strict compliance with the manufacturer's instructions and national quality control standards (Botnaru 2006).

Results were categorized as Positive or Negative, with reactive samples requiring confirmation through additional techniques according to standard protocols (Licker et al. 2020).

Statistical analysis and data visualization: Data storage, structuring, and cleaning were performed in a secure environment. Data analysis and graphical representations were carried out using the biostatistical packages *pandas* (v2.0), *scipy.stats* (v1.10), and *matplotlib* (v3.7) within the Python programming environment (version 3.10) (Gheorghe et al. 2013). Categorical variables were expressed as absolute frequencies (n) and percentages (%). 95 % Confidence Intervals (95 %CI) for prevalence rates were calculated using the Wilson score method. To evaluate the statistical significance of differences between groups, the Chi-square ($\chi 2$) test for independence was used. To compare risk by gender, the Odds Ratio (OR) was calculated alongside the 95 % Confidence Interval (95 %CI). A threshold value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

1. Overall Distribution of Results

During the analyzed period (2021–2024), a total of 5346 samples were processed and interpreted for anti-HCV antibody detection at the LABORMED Laboratory in Alexandria. Of the total evaluated sample, 5264 samples were negative (98.47 %) and 82 samples were confirmed positive, yielding an overall seroprevalence of 1.53 % (Table 1).

This value places the served population within a low-to-moderate endemicity level, remaining comparable to data reported in recent literature for the adult population in the direct-acting antiviral (DAA) era in Romania (Gheorghe et al. 2020; Sporea & Goldiș 2007).

Table 1

Overall and Gender-Stratified Distribution of Anti-HCV Test Results (2021–2024)

Test Result	Males (M) (n = 1530)	Females (F) (n = 3816)	Total cases (n=5346)	Percentage (%)
Positive	17 (1.11 %)	65 (1.70 %)	82	1.53 %
Negative	1513 (98.89 %)	3751 (98.30 %)	5264	9.47 %
Total	**1530 (100.00 %)**	**3816 (100.00 %)**	**5346**	**100.00 %**

*Note: Data are expressed as absolute frequencies (n) and percentages (%)

2. Seroprevalence Analysis by Sex

The study sample comprised 1530 males (28.62 %) and 3816 females (71.38 %), with the higher presentation rate among female patients reflecting a consistent characteristic of outpatient screening programs.

The detailed distribution of results by sex is summarized in Table 1.

Analysis of the seropositivity rate revealed slightly different values between the two sexes, though without major statistical significance: among the 1530 males tested, 17 were positive (1.11 %), whereas among the 3816 females tested, 65 were positive (1.70 %) (Figure 1).

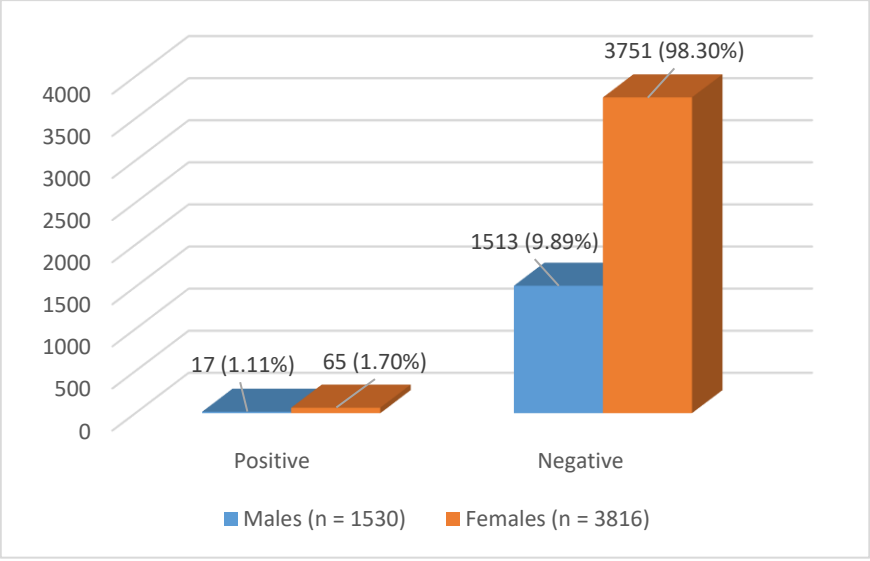


Figure 1. Anti-HCV antibody seroprevalence by sex at Labormed Alexandria (2021–2024) (χ^2 = 2,16, p = 0,1417, OR = 1,54, 95% CI: 0,90–2,64)

Application of the Chi-square test showed that the observed difference between sexes is not statistically significant (χ^2 = 2,16, p = 0,1417). Calculation of the Odds Ratio indicated a quasi-identical relative risk between sexes (OR = 1,54; 95 %CI: 0,90–2,64).

Unlike other parenteral viral hepatitis forms where male predominance is more pronounced, HCV infection distribution by sex in the general screening population tends to be more homogeneous, as exposure to iatrogenic or cosmetic/manicure/pedicure risk factors affects both sexes comparably (Gheorghe et al. 2020; Sporea & Goldiș 2007)

3. Age Group Distribution Analysis

Patient ages ranged from 1 to 92 years. For a relevant epidemiological analysis, the sample was stratified into 5 age groups: <18 years, 18–34 years, 35–49 years, 50–64 years, and ≥ 65 years (Table 2, Figure 2).

Table 2

Anti-HCV antibody seroprevalence by age group at Labormed Alexandria (2021–2024)

Age group (years)	Total tested (n)	Positive cases (n)	Seroprevalence (%)	95 %CI (%)
< 18	328	1	0.30 %	0.05 % – 1.71 %
18 – 34	849	0	0.00 %	0.00 % – 0.45 %
35 – 49	1230	5	0.41 %	0.17 % – 0.95 %
50 – 64	1559	25	1.60 %	1.09 % – 2.36 %
≥ 65	1380	51	3.70 %	2.82 % – 4.83 %
Total	**5346**	**82**	**1.53 %**	**1.24 % – 1.90 %**

*Note: Data are expressed as absolute frequencies (n) and percentages (%). 95 % Confidence Intervals (CI) were calculated using the Wilson score method.

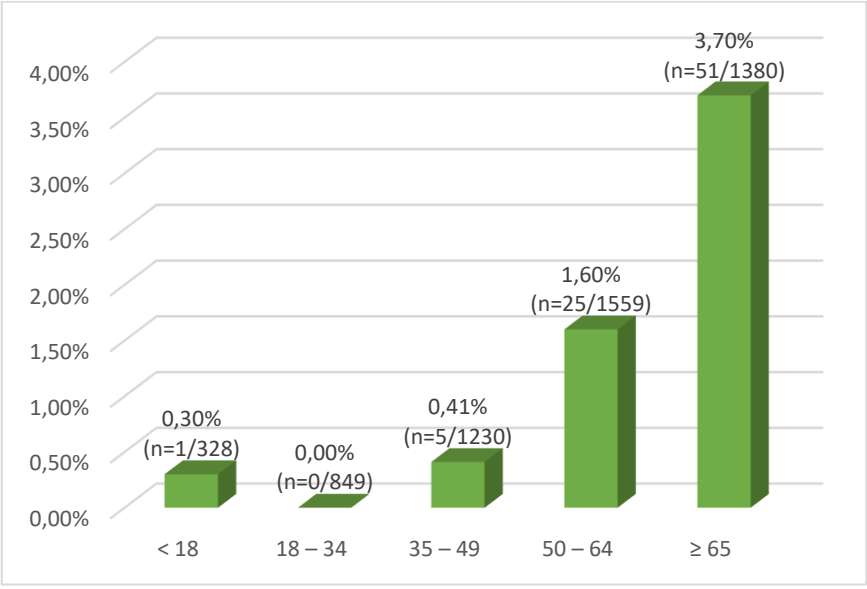


Figure 2. Anti-HCV antibody seroprevalence by age group at Labormed Alexandria (2021–2024)

The Chi-square test demonstrated an extremely strong association between age group and seropositivity rate ($\chi^2 = 69{,}61$, $p = 2{,}75 \times 10^{-14}$).

Analysis of this distribution highlights two major epidemiological pillars:

a. Low seroprevalence in the <18 age group (0.30 %, 1 positive case out of 328 children/adolescents): This finding reflects an extremely low epidemiological risk among younger generations, who benefit from modern nosocomial infection

control measures, rigorous screening of blood donors, and increased public awareness of hygiene and sanitary safety practices (Gheorghe et al. 2020).

b. Increasing prevalence trend among the adult and elderly population: The seropositivity rate rises from 0.00 % in the 18–34 age group and 0.41 % in the 35–49 age group, showing a significant acceleration in middle-aged adults aged 50–64 years (1.60 %) and reaching its peak among the elderly aged ≥ 65 years (3.70 %). This accumulation of cases in older individuals reflects historical exposure to medical or surgical procedures performed in previous decades, before modern sterilization and screening standards were implemented (Voiculescu et al. 2010).

4. Annual Dynamics of Testing and Seropositivity (2021–2024)

Evaluation of multi-year dynamics over the 4-year study period demonstrates remarkable stability in detection rates (Table 3, Figure 3).

Table 3

Annual Dynamics of Anti-HCV Testing Volume and Seropositivity at Labormed Alexandria (2021–2024)

Year	Total tests (*n*)	Positive cases (*n*)	Annual Seroprevalence (%)
2021 (Nov-Dec)	264	5	1.89 %
2022	1644	23	1.40 %
2023	1807	28	1.55 %
2024	1631	26	1.59 %
Total	**5346**	**82**	**1.53 %**

Note: Data are expressed as absolute frequencies (n) and percentages (%)

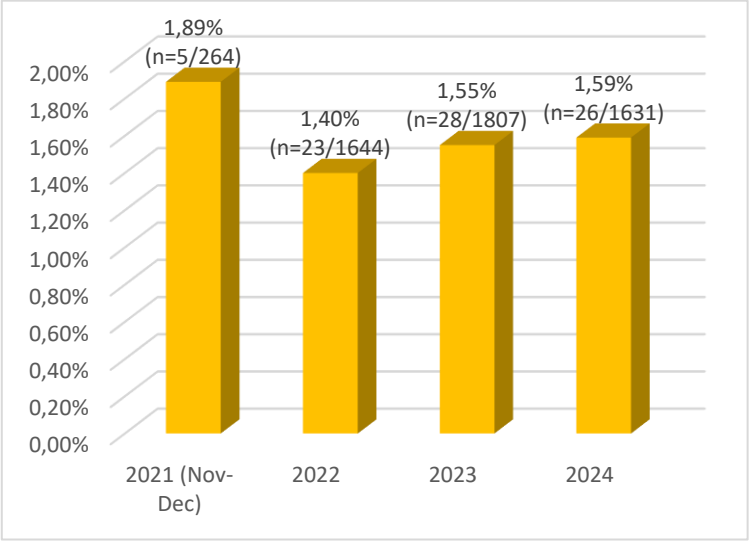


Figure 3. Annual dynamics of anti-HCV testing volume and seropositivity at Labormed Alexandria (2021–2024)

The Chi-square test for temporal trend indicated no significant year-to-year variations ($\chi^2 = 0,47$, $p = 0,9262$). The stable annual seropositivity, hovering around 1.4 %–1.6 %, underlines the stable endemic pattern of HCV infection within the served population and emphasizes the need for continuous population-based screening programs (ECDC 2023; WHO 2024).

5. Comparison with National and International Literature

The overall anti-HCV antibody seroprevalence identified in our study at Labormed Alexandria (1.53 %, 82 positive cases out of 5346 individuals tested) places Teleorman County in a moderate-to-low endemicity zone, in full agreement with current national epidemiological dynamics.

In the national context: Studies conducted in Romania during the 2000–2010 period reported an anti-HCV antibody seroprevalence of approximately 3.2 % in the general adult population, with our country considered at that time a high-prevalence area within the East European region (Gheorghe et al. 2010; Sporea & Goldiș 2007).

The 1.53 % value obtained in our sample reflects a significant reduction in population seropositivity compared to previous decades, aligning with the results of recent national screening programs and data reported by the National Center for Surveillance and Control of Communicable Diseases (Cercel et al. 2021).

In the European and international context: According to reports from the European Centre for Disease Prevention and Control (ECDC 2023), anti-HCV seroprevalence in the general population across the European Union and European Economic Area (EU/EEA) ranges between 0.5 % and 2.4 %.

The value observed in Alexandria falls squarely within this average European range. Furthermore, World Health Organization targets (WHO 2024) regarding the elimination of hepatitis C as a major public health threat are supported by the stable level identified and the absence of new cases among young individuals (0.00 % in the 18–34 age group), confirming the positive impact of modern preventive measures.

Demographic profile and risk group: The concentration of cases among the elderly (≥ 65 years, at 3.70 %) and middle-aged/older adults (50–64 years, at 1.60 %) coincides with the epidemiological profile described nationally.

This "cohort effect" confirms that the vast majority of current cases represent long-standing chronic infection acquired through historical exposures prior to the 1990s (Voiculescu et al. 2010; Gheorghe et al. 2020).

CONCLUSIONS

Overall Seroprevalence Level: In the population sample evaluated at the Labormed Alexandria laboratory between 2021 and 2024 (N = 5346), the anti-HCV antibody seroprevalence was 1.53 % (82 positive cases), indicating a moderate-to-low endemic level in Teleorman County, consistent with the European average.

Sex Distribution Homogeneity: No statistically significant differences were identified between females (1.70 %, 65/3816) and males (1.11%, 17/1530) ($\chi^2 = 2,16$, $p = 0,1417$, OR = 1,54; 95 %CI: 0,90–2,64), confirming that exposure to iatrogenic or cosmetic risk factors affects both sexes comparably.

Impact of Age and Cohort Effect: Age-stratified distribution revealed an extremely strong statistical association ($\chi^2 = 69,61$, $p = 2,75 \times 10^{-14}$), characterized by zero/minimal prevalence among young adults (0.00 % in the 18–34 age group)

and an epidemiological peak among elderly individuals (≥ 65 years, at 3.70 %). This accumulation in older age groups reflects a cohort effect driven by historical iatrogenic exposures prior to the 1990s.

Temporal Stability: The seropositivity rate maintained an exceptionally stable trend across the 4 analyzed years (1.89 % in 2021, 1.40 % in 2022, 1.55 % in 2023, and 1.59 % in 2024; $\chi^2 = 0,47$, $p = 0,9262$), confirming a constant endemic pattern and emphasizing the need to sustain active population screening to achieve the WHO objectives for eliminating hepatitis C by 2030.

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