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PREVALENCE AND EPIDEMIOLOGICAL PROFILE OF HEPATITIS B VIRUS INFECTION IN A POPULATION SAMPLE FROM SOUTHERN ROMANIA: A FOUR-YEAR RETROSPECTIVE ANALYSIS (2021–2024)

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ABSTRACT

Chronic hepatitis B virus (HBV) infection remains a major public health challenge. The present retrospective study evaluated the HBsAg seroprevalence in a sample of 5501 patients tested in Alexandria (Teleorman County) between 2021 and 2024 using rapid immunochromatographic methods. The overall seropositivity rate was 4.45 % (245 cases), with a significantly higher prevalence in males compared to females (5.62 % vs. 3.99 %, p = 0.0078; OR = 1.44). The age distribution showed a progressive increase reaching 5.68% in the 50–64 age group, whereas no positive cases were recorded in the under-18 age group, confirming the effectiveness of the national HBV vaccination program launched in 1995. Temporal dynamics revealed a slight increase in annual prevalence from 3.95 % in 2022 to 5.04 % in 2024, highlighting the necessity of continuing population screening campaigns.

INTRODUCTION

Hepatitis B virus (HBV), a member of the *Hepadnaviridae* family, is a hepatotropic virus characterized by a partially double-stranded circular DNA genome of 3200 nucleotides, enclosed within an internal nucleocapsid (core) and an outer lipoprotein envelope (Licker et al. 2020). Its antigenic structure includes the surface antigen (HBsAg), the infectivity antigen (HBeAg), the core antigen (HBcAg), and the HBx protein, the precise role of which continues to be fully elucidated (Babiuc & Dumbravă 2007).

The infection gives rise to a broad spectrum of clinical manifestations, ranging from asymptomatic, anicteric, or inapparent forms to acute hepatitis, liver cirrhosis, and hepatocellular carcinoma (Pântea 2009; Gheorghe et al. 2013). Furthermore, 10–20 % of patients with chronic infection may develop immune-mediated extrahepatic manifestations (such as glomerulonephritis, polyarteritis nodosa, cutaneous, or cardiopulmonary conditions) mediated by circulating immune complexes (Babiuc & Dumbravă 2007). Beyond hepatocytes, susceptibility to infection is also exhibited by biliary, pancreatic, renal, and cutaneous epithelial cells, as well as leukocytes (Uțu et al. 2024).

At the global level, hepatitis B demonstrates widespread endemicity (Turculeanu et al. 2013), with the World Health Organization estimating over 254

million chronically infected individuals and approximately 1.1 million deaths annually (WHO 2024). The primary transmission routes include parenteral exposure (via blood or blood products, non-sterile instrumentation, or accidental occupational injuries) (Gherasim 1999), sexual transmission, as well as non-sexual intrafamilial or institutional transmission (Dumbravă 2003). While 90 % of acute hepatitis cases follow a favorable, self-limiting course, 5–10 % progress toward chronicity (Cercel et al. 2021). In the chronic stage, the cytotoxic immune response (predominantly directed against the HBc protein and DNA polymerase) modulates viral persistence (Miftode 2008), while the persistence of viral DNA as covalently closed circular DNA (cccDNA) in hepatocytes allows potential late reactivations under conditions of severe immunosuppression (Cercel et al. 2021).

In the European epidemiological landscape, the Eastern and Southeastern regions present moderate-to-high endemicity rates (ECDC 2023). Historically, Romania was categorized as an area of medium-to-high endemicity, displaying an HBsAg seroprevalence exceeding 5–7 % in the general population prior to the 1990s, driven by nosocomial transmission and medical biosecurity limitations of that era (Voiculescu et al. 2010). The turning point in national epidemiological control was the implementation in 1995 of universal newborn vaccination as part of the National Immunization Program (Cercel et al. 2021). Nevertheless, cohorts born prior to 1995 remain exposed and represent a significant asymptomatic reservoir within the community (Gheorghe et al. 2013).

Laboratory diagnosis and population screening rely on identifying specific serological markers (HBsAg, HBeAg, anti-HBc, anti-HBe, and anti-HBs antibodies) (Moldoveanu 2012). HBsAg detection serves as the primary marker defining infection and, if persistent for more than 6 months, indicates chronic carrier status (WHO 2024). In alignment with WHO objectives to eliminate viral hepatitis as a public health threat by 2030, obtaining updated regional epidemiological data is essential for optimizing local screening programs and targeting therapeutic resources (WHO 2024; ECDC 2023).

The objective of the present study is to evaluate the HBsAg seroprevalence rate, analyze its distribution by gender and age groups, and track the multi-year dynamics of testing over a four-year period (2021–2024) within a population sample evaluated at the LABORMED Laboratory in Alexandria, Teleorman County.

MATERIAL AND METHODS

Study Design and Population Sample: A retrospective, observational study was conducted on a cohort of 5501 patients 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 HBsAg 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): 266 patients evaluated; **2022:** 1697 patients evaluated; **2023:** 1853 patients evaluated; **2024:** 1685 patients

evaluated. This temporal breakdown allowed for a comparison of population addressability rates and the identification of annual variations in HBsAg seroprevalence.

Laboratory Methodology: Qualitative determination of HBsAg in patient serum or plasma was performed using a rapid membrane-based immunochromatographic technique. The assay utilizes specific monoclonal and polyclonal anti-HBs antibodies immobilized on a nitrocellulose membrane to selectively capture HBsAg at the test line level, providing a diagnostic sensitivity exceeding 99 % and a specificity of 99.8 % (WHO 2024). Venous blood samples were collected in anticoagulant-free vacutainers with gel separators, and serum obtained by centrifugation at 3000 rpm for 10 minutes was analyzed in strict compliance with the manufacturer's instructions and national quality control standards. Results were classified into three categories: Positive, Negative, and Equivocal.

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 number of 5501 samples were processed and interpreted for HBsAg detection at the LABORMED Laboratory in Alexandria. Out of the total evaluated sample, 5253 samples were negative (95.49 %), 3 samples recorded an equivocal result (0.05 %), and 245 samples were confirmed positive, establishing an overall seroprevalence of 4.45 % (Table 1). This value places the served population within an intermediate endemicity level according to WHO criteria, being comparable to data reported in the literature for the adult population in southern Romania (Gheorghe et al. 2013; Voiculescu et al. 2010)

Table 1

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

Test Result	Males (M) (n = 1637)	Females (F) (n = 3864)	Total cases (n=5501)	Percentage (%)
Positive	92 (5.62 %)	153 (3.99 %)	245	4.45 %
Negative	1545 (94.38 %)	3708 (95.96 %)	5253	95.49 %
Equivocal	0 (0.00 %)	3 (0.05 %)	3	0.05 %
Total	**1637 (100.00 %)**	**3864 (100.00 %)**	**5501**	**100.00 %**

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

2. Seroprevalence Analysis by Sex: The sample was composed of 1,637 males (29.76 %) and 3864 females (70.24 %), with the higher addressability of female individuals being a constant characteristic of outpatient screening tests.

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

Analysis of the seropositivity rate revealed a significant difference in favor of males: out of the 1637 tested males, 92 were positive (5.62 %), whereas out of the 3864 tested females, 153 were positive (3.99 %) (Figure 1).

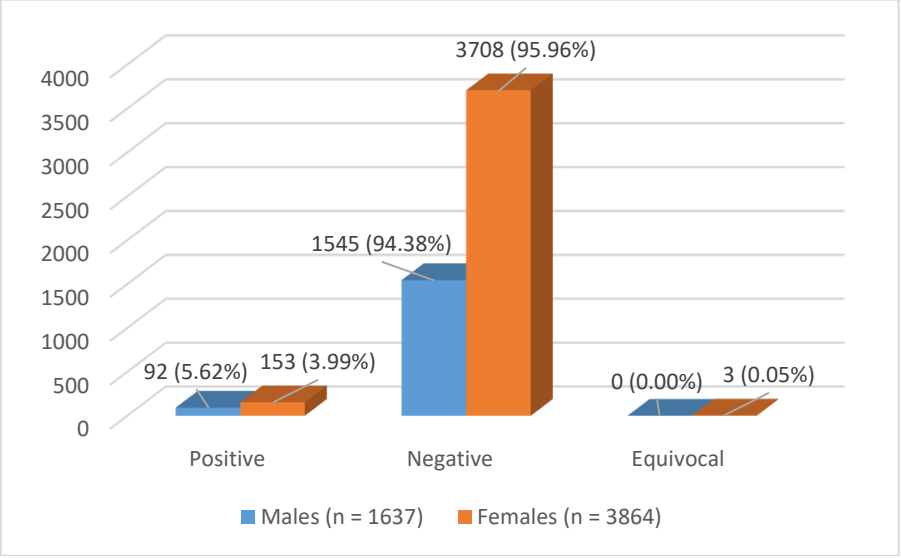


Figure 1. HBsAg seroprevalence by sex at Labormed Alexandria (2021–2024) (χ^2 = 7,06, p = 0,0078, OR = 1,44, 95% CI: 1,11–1,88)

Application of the Chi-square test confirmed that the observed difference is statistically significant (χ^2 = 7,06, *p* = 0,0078). Calculation of the Odds Ratio indicated that males in the analyzed cohort have a 44 % higher chance of being HBsAg carriers compared to females (OR = 1.44; 95 % CI: 1.11–1.88).

This male predominance is well-documented in the literature and can be attributed to both behavioral factors (occupational exposure, higher prevalence of substance use, or unassisted invasive procedures) and biological/hormonal mechanisms related to more efficient estrogen-mediated viral clearance in women (Gheorghe et al. 2013; Voiculescu et al. 2010).

3. Age Group Distribution Analysis: Patient age ranged between 1 and 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

Age-Specific HBsAg Seroprevalence at Labormed Alexandria (2021–2024)

Age group (years)	Total tested (n)	Positive cases (n)	Seroprevalence (%)	95 %CI (%)
< 18	312	0	0.00 %	0.00–2.46 %
18 – 34	1145	32	2.79 %	2.05–3.96 %
35 – 49	1580	72	4.56 %	3.90–5.91 %
50 – 64	1408	80	5.68 %	4.55–6.98 %
≥ 65	1056	61	5.78 %	3.32–6.41 %
Total	**5501**	**245**	**4.45 %**	**3.92–5.02 %**

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

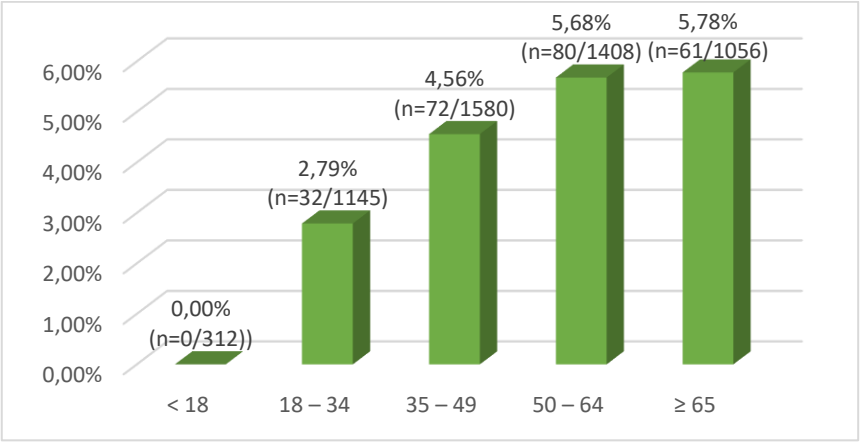


Figure 2. HBsAg seroprevalence by age group at Labormed Alexandria (2021–2024)

The Chi-square test demonstrated an extremely strong association between age group and seropositivity rate ($\chi^2 = 31{,}32$, $p = 2{,}63 \times 10^{-6}$).

Analysis of this distribution highlights two major epidemiological pillars:

a. **Complete absence of positive cases in the < 18 age group (0.00% out of 312 children/adolescents):** This finding represents direct and conclusive evidence of the positive impact of mandatory HBV vaccination at birth, introduced in Romania in 1995 (Cercel et al. 2021). Younger generations benefit from a high level of immune protection, nearly completely eliminating vertical (mother-to-child) and horizontal transmission during childhood (Gheorghe et al. 2013).

b. **Increasing prevalence trend in the adult and elderly population:** The seropositivity rate increases from 2.79 % in the 18–34 age group, reaching a peak of 5.68% in the 50–64 age group and 5.78 % in the ≥ 65 age group. This accumulation of cases in individuals over 50 years of age reflects historical exposure to parenteral

procedures performed prior to the 1990s, when sterilization techniques and transfusion screening had not yet achieved current standards of rigor (Voiculescu et al. 2010).

4. Annual Dynamics of Testing and Seropositivity (2021–2024): The evolution of the number of tests and annual prevalence at the LABORMED Laboratory was tracked throughout the four years of the study (Table 3, Figure 3)

Table 3

Annual Dynamics of HBsAg Testing Volume and Seropositivity at Labormed Alexandria (2021–2024)

Year	Total tests (*n*)	Positive cases (*n*)	Annual Seroprevalence (%)
2021 (Nov-Dec)	266	12	4.51%
2022	1697	67	3.95%
2023	1853	81	4.37%
2024	1685	85	5.04%
Total	**5501**	**245**	**4.45%**

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

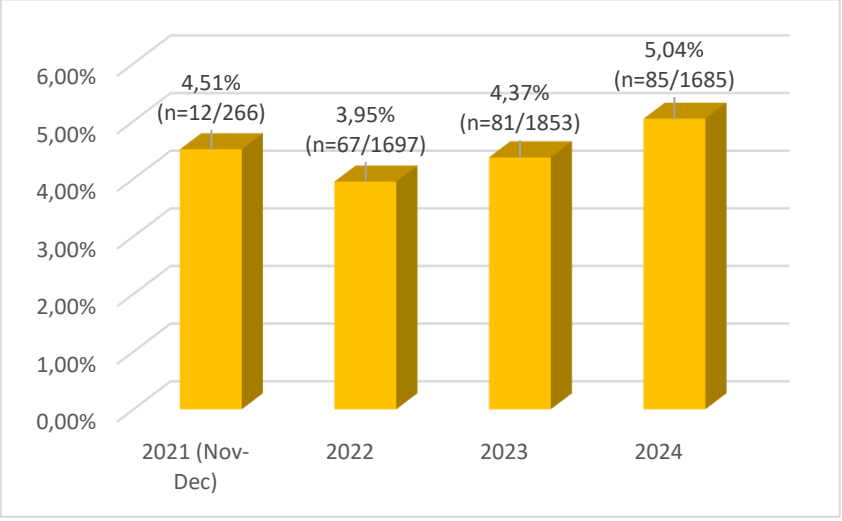


Figure 3. Annual dynamics of HBsAg testing volume and seropositivity at Labormed Alexandria (2021–2024)

A stabilization of the testing volume is observed after 2021 (which included only the last two months of the year), with an average of over 1700 evaluations per year. Between 2022 and 2024, a slight and progressive increase in annual prevalence is noted, from 3.95 % to 5.04 %. This increase does not indicate a resurgence of new acute infections, but rather reflects an intensification of outpatient addressability among the adult and elderly population toward diagnostic services in

the post-pandemic period, alongside the expansion of national viral hepatitis screening programs.

5. Comparison with National and International Literature: A comparative analysis of the results obtained at LABORMED Alexandria with data from the literature confirms their alignment with regional and global epidemiological trends:

- National level: The representative national study conducted by Gheorghe et al. (2013) reported an HBsAg seroprevalence of 4.4 % in the adult population of Romania, a value almost identical to the overall prevalence obtained in the present study (4.45 %). Furthermore, historical data summarized by Voiculescu et al. (2010) highlighted a prevalence exceeding 5–7 % prior to the 1990s, with the decrease to the current level of 4.45% being attributed to improved medical biosecurity conditions and the introduction of birth-dose vaccination.

- Gender disparity: The odds ratio (OR = 1.44) favoring the male sex found in our cohort is in full agreement with data from national (Gheorghe et al. 2013) and international reports (ECDC 2023), where chronic carrier rates in males are systematically 30–50 % higher than in females.

- **Cohort effect and vaccination:** The absence of cases in the <18 age group (0.00 %) confirms the findings of clinical studies published by Cercel et al. (2021) and Pântea (2014), which demonstrate the success of universal vaccination implemented in Romania since 1995. Conversely, the concentration of cases in individuals over 50 years of age (≥ 5.68 %) reflects the persistence of a historical endemic reservoir (Miftode 2008; Pântea 2014).

- European and international context: Reports from the European Centre for Disease Prevention and Control (ECDC 2023) place most Western European countries at a prevalence of <1 %, whereas Eastern Europe records values between 2 % and 5 %. The value of 4.45 % obtained in Teleorman County reconfirms that the Southern region of Romania remains an area of intermediate endemicity, emphasizing the need to continue active screening campaigns and linkage to specialized medical care, in accordance with World Health Organization directives (WHO 2024).

CONCLUSIONS

Endemicity Level and Overall Prevalence: The study conducted on a representative sample of 5501 patients in Teleorman County revealed an overall HBsAg seroprevalence of 4.45 %, placing the investigated population within an area of **intermediate endemicity** according to World Health Organization standards (WHO 2024), values comparable with national epidemiological data for the southern region of Romania.

Major Impact of the Vaccination Program: The complete absence of positive cases (0.00 %) among children and adolescents under 18 years of age demonstrates the indisputable efficiency and success of the national universal newborn immunization program introduced in 1995.

Vulnerability of Historical Cohorts and Elderly Population: The concentration of peak seropositivity rates in the adult and elderly age groups (5.68 % at 50–64 years and 5.78 % at ≥ 65 years) highlights the persistence of a historical viral reservoir, originating from exposure prior to the 1990 s to parenteral procedures with deficient biosecurity standards.

Gender Disparity: A statistically significant difference by gender was recorded (χ^2= 7,06, p = 0,0078), with males presenting a 44 % higher risk of being

chronic carriers compared to females (OR = 1.44), an aspect correlated with behavioral factors and immunological characteristics.

Need for Continued Screening: The multi-year dynamics (2021–2024) and the maintenance of an annual average of over 1700 tests highlight the crucial importance of maintaining and expanding active population screening programs and achieving efficient linkage to specialized treatment services, with a view to reaching the WHO targets of eliminating viral hepatitis as a public health threat by 2030.

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