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Original Research

Serological and molecular epidemiology of hepatitis D virus: a four-year single-center retrospective analysis

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Abstract

Aim: To assess the serological and molecular epidemiology of hepatitis B virus (HBV) and hepatitis D virus (HDV) infections by analyzing HBV surface antigen (HBsAg), Anti-HDV, and HDV ribonucleic acid (RNA) test results from May 2021 to May 2025 at Ordu University Training and Research Hospital.

Methods: This single-center retrospective study used Laboratory Information Management System records. Patients aged 0-100 years with at least one request for an HBsAg, Anti-HDV, or HDV RNA test were included. To minimize duplication, only the earliest record per patient for each marker was analyzed. Positivity rates were summarized descriptively and compared by sex and age using Pearson's chi-square test.

Results: Among 316,375 individuals tested for HBsAg, 8,054 (2.5%) were positive. HBsAg positivity was higher in men than in women (P = .0011). Positivity was low among those <30 years, peaked in the 40-49 age group, and declined in older age groups. Anti-HDV testing was performed in 1,483 HBsAg-positive individuals; 14 (0.94%) were anti-HDV positive, with no significant differences by sex or age (P > .05). HDV RNA was requested in 64 patients, and 3 (4.68%) were positive.

Conclusion: HBsAg positivity suggests an intermediate level of HBV endemicity in our region, whereas Anti-HDV seropositivity appears low. Because Anti-HDV and HDV RNA testing were not systematically performed in all HBsAg-positive individuals, the true burden of HDV infection may be underestimated. Standardized screening strategies among HBsAg-positive patients are warranted to better define regional HDV epidemiology.

Keywords

hepatitis B virus, hepatitis D virus, seropositivity

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Introduction

Hepatitis delta virus (HDV) is a defective, single-stranded ribonucleic acid (RNA) virus that is dependent on hepatitis B virus (HBV) and requires the presence of HBV surface antigen (HBsAg) in hepatocytes for replication.[1] Accordingly, HDV infection occurs only in individuals infected with HBV and manifests clinically in two distinct forms: coinfection or superinfection. While coinfection is generally associated with a more favorable prognosis, superinfection is characterized by a markedly higher likelihood of chronicity and has been linked to rapid fibrosis progression, cirrhosis, and a substantially increased risk of hepatocellular carcinoma.[2,3]

According to large-scale meta-analyses, approximately 0.16% of the global population (around 12 million people) is estimated to be infected with HDV, and the mean Anti-HDV prevalence among HBsAg-positive individuals is approximately 4.5%.[3] However, HDV infection demonstrates a geographically heterogeneous distribution, with the Mediterranean basin, the Middle East, Africa, and parts of South America recognized as highly endemic regions.[3] Although Türkiye is considered a region of intermediate endemicity, epidemiological data indicate pronounced intranational variation in HDV seroprevalence, with substantially higher rates reported in Eastern and Southeastern Anatolia compared with western regions.[4]

Current international guidelines recommend that all HBsAg-positive individuals be screened for Anti-HDV at least once, and that high-risk populations be reassessed at regular intervals.[5] Serological assays (Anti-HDV) are used for screening, whereas molecular testing (HDV RNA), regarded as the gold standard for identifying active infection, is used to confirm viremia.[2] To date, no globally approved antiviral therapy with high efficacy is available for HDV infection; therefore, the most effective strategy for prevention remains control of HBV infection and vaccine-induced immunity.[1,2]

Given the severity of HDV-related clinical outcomes, the rapid progression of its natural course, and the marked regional differences observed in Türkiye, obtaining up-to-date seroprevalence data is epidemiologically crucial.

The objective of this study was to evaluate the serological and molecular epidemiology of HBV and HDV infections in our region using HBsAg, Anti-HDV, and HDV RNA test results collected over a four-year period.

Materials and Methods

This single-center retrospective study evaluated HBsAg, Anti-HDV, and HDV RNA test results performed at the Microbiology Laboratory of Ordu University Training and Research Hospital between May 2021 and May 2025, using data extracted retrospectively from the Laboratory Information Management System. All patients aged 0–100 years who had at least one of these three tests requested during the study period were included.

In routine clinical practice, Anti-HDV and HDV RNA testing were requested for patients with HBsAg positivity at the physicians' discretion. To prevent repeated test orders from influencing the analyses, records were merged using the patient identification number, and for each marker, the chronologically first test request date was retained. Subsequent repeat requests for the same marker were excluded from analysis. Because Anti-HDV and HDV RNA testing was initiated at the discretion of clinicians, estimates such as Anti-HDV seropositivity and HDV RNA positivity were calculated and reported within the subpopulation in whom the respective test was requested, rather than across the entire HBsAg-positive cohort.

HBsAg was measured using an electrochemiluminescence immunoassay on the Roche Cobas e601 analyzer (Roche Diagnostics, Mannheim, Germany). Anti-HDV testing was performed at an external reference laboratory using the Triturus fully automated ELISA system (Diagnostics Grifols S.A., Barcelona, Spain). HDV RNA analyses were conducted using the Montania 4896 real-time polymerase chain reaction (PCR) platform (Anatolia Geneworks, Istanbul, Türkiye).

Ethical Approval

This study was approved by the Ethics Committee of Ordu University, Faculty of Medicine (Date: 09.07.2025, Decision No: 235).

Statistical Analysis

Data were summarized using descriptive statistics. Categorical variables are presented as numbers and percentages (%). Differences in the distribution of HBsAg and Anti-HDV positivity were assessed using the Pearson chi-square test; Fisher's exact test was applied when expected cell counts were <5. Statistical analyses were performed using IBM SPSS Statistics (Version 25.0; IBM Corp., Armonk, NY, USA), and a two-sided P value < 0.05 was considered statistically significant.

Reporting Guidelines

This study was reported in accordance with the STROBE guideline.

Results

During the study period, HBsAg testing was performed on 316,375 patients, of whom 164,499 (52.0%) were female. Overall, 8,054 tests (2.5%) were HBsAg-positive, and 50.1% of the positive cases were female. Among 1,483 HBsAg-positive individuals who underwent Anti-HDV testing, 14 (1.0%) were found to be Anti-HDV positive. Of the 64 HDV RNA tests evaluated, 3 (4.68%) were positive.

With respect to testing patterns, among the 8,054 patients with HBsAg positivity, 791 (9.82%) had an Anti-HDV test requested, and 24 (0.30%) had an HDV RNA test requested. Among the 1,483 patients in whom Anti-HDV testing was requested, HBsAg records could be matched for 1,460 patients, whereas HBsAg data were not available for 23 patients. Among the 64 patients for whom HDV RNA testing was requested, 38 also had an Anti-HDV test record; of these, 5 (13.2%) were Anti-HDV-positive. No Anti-HDV record was identified for 26 patients (40.6%). Of the three patients with HDV RNA positivity, one had a positive Anti-HDV result.

By sex, HBsAg positivity was 2.64% (95% CI: 2.56–2.72) in males and 2.46% (95% CI: 2.38–2.53) in females. HBsAg positivity was significantly higher in males compared with females (95% CI: 1.03–1.13; P = .0011).

Across age groups, HBsAg positivity differed significantly (P < .001). The lowest seropositivity rates were observed in the 0-19 age group (0.25%; 95% CI: 0.21-0.32) and the 20-29 age group (0.95%; 95% CI: 0.89-1.03), indicating markedly lower HBsAg positivity in individuals younger than 30 years. Seropositivity increased to 3.12% in the 30-39 age group, peaked at 4.67% in the 40-49 age group, and remained at 4.32% in the 50-64 age group. A decreasing trend was observed in older age groups, with seropositivity of 2.36% in those aged 65-74 years and 1.40% in those aged ≥ 75 years (Table 1).

Among 1,483 HBsAg-positive individuals who underwent Anti-HDV testing, 14 (0.94%) were found to be Anti-HDV positive.

Table 1 Seropositivity of HBsAg Positivity by Sex and Age Groups

Variable	Group	Total (*N*)	Seropositivity *n* (%)	95% CI	X^2	*P* value
Sex	Male	151876	4011 (2.64)	(2.56-2.72)	10.68	.0011*
	Female	164499	4043 (2.46)	(2.38-2.53)	10.68	
	Total	**316375**	**8054 (2.5)**	**(2.49-2.60)**		
Age, y	0-19	35728	92 (0.25)	(0.21-0.32)		< .001*
	20-29	68993	659 (0.95)	(0.89-1.03)		
	30-39	44139	1377 (3.12)	(2.96-3.29)		
	40-49	42619	1989 (4.67)	(4.47-4.87)		
	50-64	63007	2724 (4.32)	(4.17-4.48)		
	65-74	36178	852 (2.36)	(2.20-2.52)		
	75+	25701	361 (1.40)	(1.27-1.56)		
	Total	**316375**	**8054 (2.5)**	**(2.49-2.60)**		

**Pearson's chi-square test was used. A P value < .05 was considered statistically significant*

When stratified by sex, Anti-HDV positivity was 0.63% (95% CI: 0.27-1.47) in males and 1.30% (95% CI: 0.68-2.45) in females; the difference was not statistically significant (*P* = .187).

Across age groups, Anti-HDV positivity was very low, with no positive cases observed in several strata. Specifically, no anti-HDV-positive cases were identified in the 0-19, 20-29, 65-74, and ≥ 75 age groups. Anti-HDV positivity was 0.33% (95% CI: 0.06-1.85) in the 30-39 age group, 1.51% (95% CI: 0.69-3.26) in the 40-49 age group, and 1.37% (95% CI: 0.66-2.79) in the 50-64 age group. The overall association between age group and Anti-HDV positivity was not statistically significant (*P* = .406) (Table 2).

Table 2 Seropositivity of Anti-HDV Positivity by Sex and Age Groups

Variable	Group	Total (*N*)	Seropositivity *n* (%)	95% CI	X^2	*P* value
Sex	Male	789	5 (0.63)	(0.27-1.47)	1.74	.187
	Female	694	9 (1.30)	(0.68-2.45)	1.74	
	Total	**1483**	**14 (0.94)**	**(0.56-1.58)**		
Age, y	0-19	13	0	(0-0)	6.15	.406
	20-29	114	0	(0-0)	6.15	
	30-39	302	1 (0.33)	(0.06-1.85)	6.15	
	40-49	397	6 (1.51)	(0.69-3.26)	6.15	
	50-64	512	7 (1.37)	(0.66-2.79)	6.15	
	65-74	113	0	(0-0)	6.15	
	75+	32	0	(0-0)	6.15	
	Total	**1483**	**14 (0.94)**	**(0.56-1.58)**		

Pearson's chi-square test was used for statistical analyses, and a P value < .05 was considered statistically significant

Pearson's chi-square test was used for statistical analyses, and a *P* value < 0.05 was considered statistically significant.

HBsAg and Anti-HDV positivity rates were evaluated by year across the four-year study period (2021-2025). The HBsAg positivity rate was 2.60% in 2021-2022, 2.47% in 2022-2023, 2.57% in 2023-2024, and 2.52% in 2024-2025. The corresponding Anti-HDV positivity rates were 2.51%, 0%, 1.18%, and 2.02%, respectively. The distribution of HBsAg and Anti-HDV positivity rates across the study periods is presented in Figure 1.

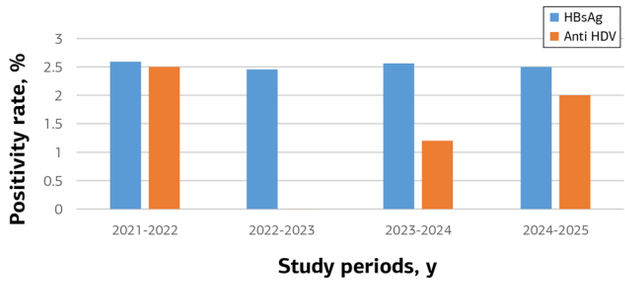

Figure 1. *Annual HBsAg and Anti-HDV Positivity Rates*

Discussion

In the present study, the HBsAg positivity rate was 2.5%, which is comparable to global prevalence estimates. Worldwide, HBsAg positivity has been reported at approximately 3.2%; however, some meta-analyses have documented higher rates reaching 5.8%.[6] In a study by Al-Busafi et al., this variability was attributed to differences in geographic distribution, age structure, and vaccination policies.[7] The proximity of our estimate to the global average is also consistent with national data indicating an intermediate level of HBV endemicity in our region.

We observed that HBsAg positivity was low among individuals younger than 30 years, peaked in the 40-49 age group, and declined in older age strata. This pattern suggests that HBV epidemiology may be influenced more by birth cohort–specific risk profiles than by aging per se. Following the introduction of the national hepatitis B vaccination program in Türkiye in 1998, HBsAg positivity has been reported to decline markedly among cohorts born after program implementation, with prevalence falling below 1% in younger age groups.[8] Similarly, global studies have shown that HBsAg prevalence tends to be higher among middle-aged

adults and to decrease in older age groups, potentially reflecting cumulative HBsAg seroclearance and HBV-related mortality.[9]

The higher HBsAg positivity observed among males is also consistent with the literature. Numerous epidemiological studies have reported significantly higher HBV positivity rates in men than in women, which may be related to both hormonal and behavioral factors. In a large-scale meta-analysis, HBV positivity in males was approximately 1.3-1.6 times higher than in females.[10] Consistent with this, a population-based study from China reported HBsAg positivity rates of 6.24% in men and 5.88% in women.[11] Our findings follow the same trend and suggest that HBV infection may remain more prevalent among males in our setting.

In our study, Anti-HDV seropositivity was 0.94%; however, this estimate may not fully reflect the true prevalence, particularly given the marked limitation in Anti-HDV testing coverage. Indeed, among 8,054 patients with HBsAg positivity, only 791 (9.82%) underwent Anti-HDV testing, potentially leading to an underestimation of the seropositivity rate. In Europe, Anti-HDV prevalence is generally reported to range between 1% and 5%, consistent with a low-to-intermediate endemicity profile similar to that of our region.[12] In Türkiye, recent meta-analyses assessing the national HDV burden have reported Anti-HDV positivity rates of 5.05% among outpatients and 3.37% among blood donors.[13] Taken together, these data suggest that the low seroprevalence observed in our study likely reflects limited screening and the selective ordering of Anti-HDV testing primarily in the presence of clinical suspicion or perceived risk. Therefore, implementing a more comprehensive screening strategy among HBsAg-positive individuals may help to more accurately define the true prevalence of HDV infection.

The literature does not provide a uniform pattern regarding sex differences in Anti-HDV seropositivity. Studies from different countries have reported higher rates in males in some series and in females in others, while many have found no statistically significant difference. For example, recent reviews and studies have described cohorts with higher seropositivity in males, as well as populations in which HDV coinfection is relatively more frequent in females.[14] Such variability has been attributed to sex specific behavioral risks, differences in the target population studied (e.g., intravenous drug use, high-risk sexual behavior, comorbid HIV infection), and heterogeneity in healthcare-seeking behaviors and screening practices.[15] In our study, the higher Anti-HDV positivity observed in females is therefore more likely to reflect regional healthcare utilization patterns, physician ordering practices, and the frequency of healthcare encounters among HBsAg-positive women rather than a biological sex effect. Accordingly, sex-based differences should be interpreted with caution, taking into account both sample composition and the selective nature of test ordering.

Although the number of anti-HDV-positive cases in our cohort was limited, the clustering of seropositive individuals in middle-aged groups, where HBsAg seropositivity is higher, is expected epidemiologically, given that HDV infection occurs only in HBsAg-positive individuals. Consistent with this, previous reports indicate that Anti-HDV positivity or HDV coinfection is more frequently identified among middle-aged adults in settings with higher HBsAg prevalence, whereas it is less common in younger age groups.[12,15]

In our study, the HDV RNA positivity rate was 4.68%, which may be attributable less to the absence of active replication among Anti-HDV positive individuals and more to selective testing practices and limited utilization of HDV RNA assays. Current guidelines recommend HDV RNA testing to confirm active infection in individuals with Anti-HDV positivity, as Anti-HDV alone does not establish ongoing replication.[5] Moreover, substantial proportions of viremia have been reported among Anti-HDV-positive cohorts; for instance, a multicenter long-term follow-up study documented HDV viremia in 64.3% of patients during follow-up, and clinical cohort data have suggested that active infection can be demonstrated in approximately two-thirds of Anti-HDV-positive individuals.[16,17] In this context, the limited number of patients undergoing HDV RNA testing, either concurrently with Anti-HDV or based on clinical suspicion, may have contributed to the low HDV RNA positivity observed in our dataset.

From an epidemiological standpoint, the most important implication of our findings is that HBV infection remains a substantial public health concern in our region, whereas the HDV burden appears to be low to intermediate. Global evidence indicates that, in certain high-risk regions, HDV substantially worsens the clinical course of HBV infection; thus, the relatively low rates observed in our setting may be attributable to the geographic heterogeneity of HDV distribution.[18] Given the well-established role of HDV in aggravating HBV-related liver disease, ongoing surveillance of regional HDV epidemiology is essential for informing clinical management strategies.[16]

Future studies should evaluate Anti-HDV and HDV RNA testing using a more standardized diagnostic algorithm, incorporate clinical correlates, and include larger population-based cohorts to better characterize regional epidemiology. In addition, updating national HDV screening strategies and routinely reporting regional laboratory data would contribute to a more accurate estimation of the true HDV burden.

Limitations

This study has a single-center, retrospective design; therefore, the generalizability of the findings to other regions and broader populations is limited. Because the analyses relied exclusively on Laboratory Information Management System records, we were unable to link laboratory results with clinical findings, diagnoses, risk factors (e.g., intravenous drug use, high-risk sexual behavior), treatment status, or disease course; consequently, the clinical impact and severity of HDV infection could not be assessed. In addition, because Anti-HDV and HDV RNA testing were not implemented as routine screening for all HBsAg-positive individuals but were instead requested at the physician's discretion based on clinical suspicion, the estimated rates of Anti-HDV seropositivity and HDV RNA positivity may be subject to selection bias and could underestimate the true prevalence. The limited number of HDV RNA test requests and the absence of matched records across markers for some patients may also have reduced the robustness of the cross-marker analyses. Accordingly, while our findings provide valuable insight into the regional burden of HDV, they should be corroborated by multicenter, preferably prospective studies conducted using standardized screening algorithms.

Conclusion

This study provides updated laboratory-based evidence on the seroepidemiological profile of HDV in our region. Over the four-year period, an HBsAg positivity rate comparable to global averages indicates that HBV infection remains a significant public health problem locally. The Anti-HDV positivity rate of 0.94% suggests a low-to-intermediate level of HDV endemicity. However, because Anti-HDV testing was not performed in all HBsAg-positive individuals, the observed Anti-HDV positivity may underestimate

the true seroprevalence. Similarly, HDV RNA testing, requested in a limited number of cases either concurrently with Anti-HDV testing or based on clinical suspicion, may have led to an underestimation of the true prevalence of HDV viremia. Larger population-based and prospective studies are warranted to improve the understanding of HDV epidemiology and to inform the development of appropriate national screening policies.

Declarations

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

Informed Consent

Informed consent was waived due to the retrospective design of the study.

Data Availability

The datasets used and/or analyzed during the current study are not publicly available due to patient privacy reasons but are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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None.

Author Contributions (CRediT Taxonomy)

Conceptualization: H.Ö.K., S.Y.
Methodology: H.Ö.K., S.Y.
Investigation: H.Ö.K., Y.E.İ.
Data Curation: Y.E.İ., S.Y.
Formal Analysis: S.Y.
Writing – Original Draft: H.Ö.K.
Writing – Review & Editing: S.Y., M.K.C.
Supervision: M.K.C.

AI Usage Disclosure

Artificial intelligence tools were used only for language editing and improving readability. No AI tool was used for data analysis, interpretation of results, or generation of scientific content. The authors take full responsibility for the content of the manuscript.

Abbreviations

Anti-HDV: Anti-hepatitis d virus
CI: Confidence interval
HBsAg: Hepatitis b surface antigen
HBV: Hepatitis b virus
HDV: Hepatitis d virus
PCR: Polymerase chain reaction
RNA: Ribonucleic acid
STROBE: Strengthening the reporting of observational studies in epidemiology

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