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Original Article

Decreased risk of nosocomial transmission of hepatitis B and C viruses among hemodialysis patients in Southern Bulgaria

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Abstract

Introduction: Hepatitis B (HBV) and (HCV) virus infections represent a nosocomial risk in hemodialysis (HD) patients. We aimed to evaluate the risk among adult HD patients in southern Bulgaria.

Methodology: A prospective cohort study included 225 patients from three HD centers between January 2020 and June 2022. HBV and HCV infections were characterized by serological and virological markers determined through enzyme-linked immunosorbent assay and polymerase chain reaction.

Results: HBV infection was detected in 13 patients and HCV in 15. Ten of the hepatitis patients died of non-liver-related complications. Sustained virological response (SVR) was confirmed in five HCV-infected patients previously cured with direct-acting antivirals (DAAs). Five patients were viremic. Three of them achieved SVR after DAAs, and two refused treatments. A decrease in HCV viremia prevalence (2.22% versus 0.89%) was recorded (p = 0.15). Virological suppression was confirmed in four HBV-infected patients treated with nucleos(t)ide analogs. Two patients were not eligible for antivirals. Decreased HBV viremia prevalence (2.7% versus 0.89%) was recorded (p = 0.15). Among HBV surface antigen (HBsAg)-negative patients, HBV vaccination coverage was 62.74% (133/212) and higher in 2 HD centers (128/137; 93.43%). Nevertheless, one-third of participants (34/112; 30.36%) were susceptible to HBV. Twenty-four vaccinees (24/112; 21.43%) had acquired natural immunity but remained at risk of reactivation in case of immunosuppression. HBV DNA was detected in eight HBsAg-negative patients, resulting in a prevalence of 7.14% (8/112) for occult HBV infection.

Conclusions: The study reveals a downward trend in HBV and HCV viremia prevalence among HD patients. To further reduce the risk of nosocomial transmission, vaccination for hepatitis B requires updating.

Key words: Hepatitis B; hepatitis C; hemodialysis; nosocomial transmission; viremia.

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Introduction

Viral hepatitis, especially those caused by hepatitis B virus (HBV) and C viruses (HCV), are a significant cause of morbidity and mortality. Chronic hepatitis B and C account for an estimated 1.3 million deaths globally, including liver cancer and cirrhosis, exceeding the number of deaths caused by human immunodeficiency virus (HIV) infection, tuberculosis, or malaria in 2022 [1].

Both HBV and HCV are treatable infections but with different efficacies. The introduction of direct-acting antiviral drugs (DAAs) has significantly improved the cure rate for HCV infection in most patients within a very short duration.

HBV infection can be suppressed through the application of specific antiviral agents, which require long-term treatment and monitoring. Universal infant immunization with the HBV vaccine is the most effective means of preventing acute and chronic HBV infection [2].

In 2016, the World Health Organization (WHO) introduced the Global Hepatitis Strategy aiming to reduce new infections by 90% and deaths by 65% by 2030, compared to the 2015 baseline [3]. Unfortunately, as of March 2023, no countries are on track to achieve WHO elimination targets for HBV [4]. This is in contrast to HCV infection, for which 9 countries are on track to meet WHO elimination targets [5].

Given the complexity and scale of HCV elimination, the European Association for the Study of the Liver has proposed a micro-elimination approach as a valid alternative [6]. It has focused on smaller unique, disproportionately affected but often overlooked groups, such as injecting drug users (IDUs), prisoners, men who have sex with men (MSM), sex workers, transgender or gender-diverse populations, and HIV-positive persons. These individuals, along with hemodialysis (HD) recipients, have been often diagnosed with HCV infection and most of them are under regular medical care. Scaling up testing and treatment uptake to achieve HCV micro-elimination for these high-risk special populations may facilitate global HCV elimination by 2030 [7].

The susceptibility to acquiring blood-borne infections, particularly viral hepatitis during HD treatment, is related to numerous factors for both the patients and the procedure. Patients' impaired cellular immunity increases their susceptibility to infection and risk for chronicity. In addition, the HD procedure results in more parenteral exposures through the extracorporeal blood circuit for a prolonged period. Moreover, HD patients may require blood transfusions (although rarely since the introduction of erythropoietin), frequent hospitalizations, and surgery for vascular access, which increase the opportunity for nosocomial transmission. Finally, HBV vaccination in such patients is less effective, and protective immunity is short-lived [2].

HD recipients are also at high risk of occult HBV infection **(**OBI**),** a phase in the natural history of the infection, which often represents a very low replicative state of the virus. OBI is defined as the presence of replication-competent HBV DNA at a low level (< 200 IU/mL) in the serum and/or in the liver of patients who are hepatitis B surface antigen (HBsAg) negative. Most patients tested positive for hepatitis B core antibody (anti-HBc) and/or hepatitis B surface antibody (anti-HBs), i.e., seropositive OBI, and very few were seronegative [8]. Globally, the prevalence of OBI is estimated to be 0.82% and it is higher in patients with HBV/HIV coinfection (16%) and HD patients (4%) [9]. In these patients, HBV DNA is only intermittently detected at low concentrations in the blood. The lowest limit of detection (LOD) of the majority of the currently available commercial HBV DNA assays is 12-20 IU/mL, and the result of these assays usually confirms the presence of OBI. OBI is associated with a risk of reactivation, transmission, and liver disease progression [10].

In Bulgaria, there are very few reports on HCV and HBV infections among HD recipients. One of the earliest studies revealed that more than half of the HD patients tested were positive for hepatitis C antibody (anti-HCV) [11]. In 2011, the anti-HCV prevalence rate decreased to 11% [12]. The rate of HBsAg prevalence among hemodialysis patients in 2008 varied from 6.2% to 3.3% [13]. All these studies used only serological testing. There are no data on the actual status of these infections among HD patients. Therefore, we initiated the present study aiming to characterize HCV and HBV infections and to evaluate the risk of nosocomial transmission among adult HD patients in southern Bulgaria. According to the Bulgarian guidelines for HD treatment, all HD recipients are screened annually for HBV, HCV, and HIV by testing for HBsAg, anti-HCV, and anti-HIV, respectively [14]. In line with the same guidelines, HBsAg-negative patients are immunized with a standard three-dose regimen of recombinant HBV vaccine, with boosting dependent on the immunological response starting one year after the vaccination series completion.

Methodology

Study population and sample collection

The study was carried out between January 2020 and June 2022 and included 225 adult patients undergoing maintenance HD in three HD centers in southern Bulgaria: two in the Plovdiv region and one in the Pazardzhik region. These centers comprised one public hospital-based (n = 80) and 2 private independent centers (n = 145).

The inclusion criteria for enrollment in the study were: patients with end-stage kidney disease (ESKD) on chronic HD treatment who were present at the time of the study in the respective HD center.

The exclusion criteria were: patients transferred to another HD center during the study period, transient HD patients, those with an acute medical indication

requiring HD treatment, and patients undergoing peritoneal dialysis.

All 225 patients were tested for hepatitis E virus infection, and none of them were found to have active HEV infection [15].

Relevant data, including age, gender, etiology of ESKD, comorbidity status, duration of HD, details on blood transfusion, organ transplantation, and HBV vaccination history, were retrieved from the patients' medical records or collected through a questionnaire. Upon enrollment, HBV infection (HBsAg) was present in 13 patients (5.7%), 15 patients (6.6 %) were anti-HCV positive, and one (0.4%) had HIV infection, who subsequently initiated HD treatment due to diabetic nephropathy. Five patients with HCV infection had been cured with DAAs before enrolment in the study. A cured HCV infection is referred to as sustained virological response (SVR) and is defined as the absence of detectable HCV RNA in the blood at least 12 weeks after treatment is completed (SVR12) [16]. Another three patients had already developed liver cirrhosis - one was alcohol-related and the other two were HCV- and HBV-related, respectively.

Table 1. Baseline demographic and clinical data of patients (n = 225).

Variable	Hemodialysis Patients (n = 225)
Age (years), median (IQR)	64 (52.5;71.5)
Gender, *n (%)*	
Male	126 (56.0)
Female	99 (44.0)
Duration of dialysis, (months), median (IQR)	48 (24;72)
Diseases that led to hemodialysis, *n (%)*	
Autosomal dominant polycystic kidney disease	34 (15.2)
Chronic tubulointerstitial nephritis	34 (15.2)
Chronic glomerulonephritis	33 (14.7)
Chronic calculous pyelonephritis	25 (11.1)
Diabetic nephropathy	21 (9.3)
Hypertensive kidney disease	18 (8.0)
Bilateral hydronephrosis	10 (4.4)
Chronic nephritic syndrome	10 (4.4)
Other diseases	40 (17.7)
Blood transfusion, *n (%)*	132 (58.7)
Tattoos, *n (%)*	14 (6.22)
Previous kidney transplantation, *n (%)*	9 (4.0)
Vascular access, *n (%)*	
Arteriovenous fistula	121 (53.8)
Permanent tunneled catheter	99 (44.0)
Vascular prosthesis	5 (2.2)
Hepatitis B vaccination (3 doses), *n (%)*	62.74% (133/212)
Viral hepatitis markers, *n (%)*	
HBsAg	13 (5.3)
anti-HCV	15 (6.6)
Viremia, *n* (%)	
HCV viremia prevalence	5 (2.2)
HBV viremia prevalence	6 (2.7)

Variables are expressed as the number (%) or the median (IQR). HD: hemodialysis; HBVv: hepatitis B virus viremia; HCVv: hepatitis C virus viremia; HBsAg: hepatitis B surface antigen; IQR: interquartile range.

Serological analysis

HBV infection (HBsAg, hepatitis B e antigen (HBeAg), anti-HBc, anti-HBs, and hepatitis B e antibody[anti-HBe]) were tested by an enzyme-linked immunosorbent assay (ELISA [DiaPro, Milan, Italy]). Anti-HBs ≥ 10 mIU/mL was considered protective [17].

HCV infection was determined by detecting anti-HCV by enzyme-linked immunosorbent assay (ELISA [DiaPro, Milan, Italy]). A Western blot test (HCV Blot 3.0, MP Diagnostics, Eschwege, Germany) was used to differentiate resolved HCV infection. Only samples with confirmed positive results in both tests were considered HCV-positive.

Nucleic acid detection and sequencing

Quantification of HBV DNA and HCV RNA was performed by real-time PCR (Cobas TaqMan HBV test, v 2.0, USA, with a 9 IU/mL LOD and Cobas TagMan HCV test, v 2.0, USA, with a 15 IU/mL LOD). OBI was investigated in cases where HBV DNA was present despite a negative HBsAg result. HCV RNA was monitored in all anti-HCV positive and HCV RNA negative patients.

HBV DNA and HCV RNA were extracted automatically from 0.4 mL serum (Exiprep DX16, Bioneer). Genotyping of HBV (DeepChek assay RT genotyping and drug resistance V1-RUO, ABL S.A., Luxemburg) and HCV (DeepChek assay NS5B/5'UTR genotyping V2, ABL S.A., Luxemburg) was done by next-generation sequencing (NGS). Sequencing was performed on the MiSeq sequencer with the MiSeq Reagent Kit, v3 (Illumina, San Diego, CA, USA) using Illumina paired-end sequencing protocols. The NGS outputs were configured and analyzed by the DeepChek® Software (CE-IVD) on HBV and HCV modules.

All analyses were conducted according to the manufacturer's instructions.

Statistical analysis

Continuous variables were expressed as median values (interquartile range, IQR). Categorical variables were expressed as absolute numbers and percentages. The statistical analysis was performed using Statistical Package for the Social Sciences SPSS v. 26 for Windows (IBM Corp. Released 2019. IBM Corp., Armonk, NY, USA). A p value < 0.05 was considered statistically significant.

Ethical statement

The study was approved by the University Ethical Committee of the Medical University of Plovdiv

(protocol No. 2/8-9 April 2020). All patients provided their written informed consent.

Results

Patients' characteristics

The median age of the 225 patients was 64 years (IQR 52.5;71.5), with a slight predominance of men (n = 126, 56%). The median duration of HD treatment before study enrollment was 48 months (IQR 24;72). Polycystic kidney disease and chronic tubulointerstitial nephritis (15.11% each) and chronic glomerulonephritis (14.66%) were the most common diseases that led to ESKD and HD. The baseline demographic and clinical characteristics of participants are presented in Table 1.

Positive serological results confirmed HBV infection in 13 patients, HCV infection in 15 patients, and HIV infection in one patient. There were no newly diagnosed HBV and HCV infections, nor transfer of infected patients from other HD units. No HCV/HBV/HIV and HBV/HCV co-infected patients were detected.

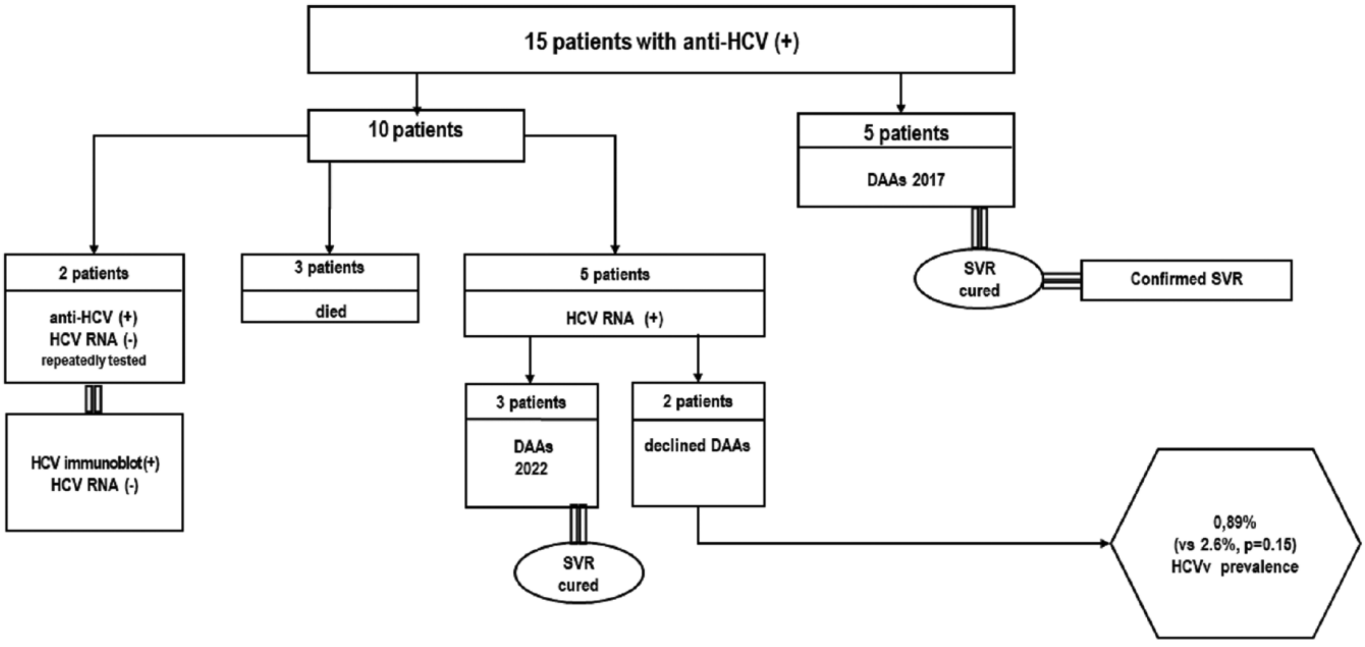
HCV infection testing results

Among the anti-HCV-positive patients (n = 15), SVR was confirmed in five patients previously cured with DAAs (dasabuvir and ombitasvir/paritaprevir/ritonavir) 6 years before their enrollment in the study (Figure 1). Of note, three of these were pretreated with lamivudine because of an underlying OBI. Among the remaining 10 anti-HCV-positive patients, three died of non-liver-related complications during the study. Five of the seven anti-HCV-positive patients were HCV RNA-positive (HCV RNA varied between 82661 and 236 276 IU/mL). Three out of the five HCV RNA-positive samples were adequate for further sequencing, which revealed HCV genotype 1b. Of these five HCV RNA-positive patients, three were referred to a gastroenterologist for antiviral treatment. They received pan-genotypic DAAs treatment (glecaprevir-pibrentasvir) for 2 months and achieved an SVR12 in 2022. The other two HCV RNA-positive cases suffered severe comorbidity (one with alcoholic cirrhosis and the other with hypothyroidism on high-dose levothyroxine). They refused DAAs for personal reasons and remained HCV RNA positive till the end of the study. Regardless of these two patients, a decreased HCV viremia (HCVv) prevalence was observed (5/225 [2.22%] in 2020 versus 2/225 [0.89%] in 2022) ($p = 0.15$).

Interestingly, three consecutive tests detected no HCV RNA in two anti-HCV-positive cases, although the latter had received no DAAs treatment. Immunoblot testing confirmed the positive anti-HCV results. Both patients had started HD treatment in their mid-twenties. We assumed these patients had cases of spontaneously cleared HCV infection.

Finally, the remaining 210 anti-HCV-negative patients tested negative for HCV RNA.

Figure 1. Hemodialysis patients with anti-HCV positive results (n = 15).



HCV: hepatitis C virus; anti-HCV: hepatitis C antibody; SVR: sustained virological response; DAAs: direct-acting antivirals; HCVv: HCV viremia

HBV infection testing results

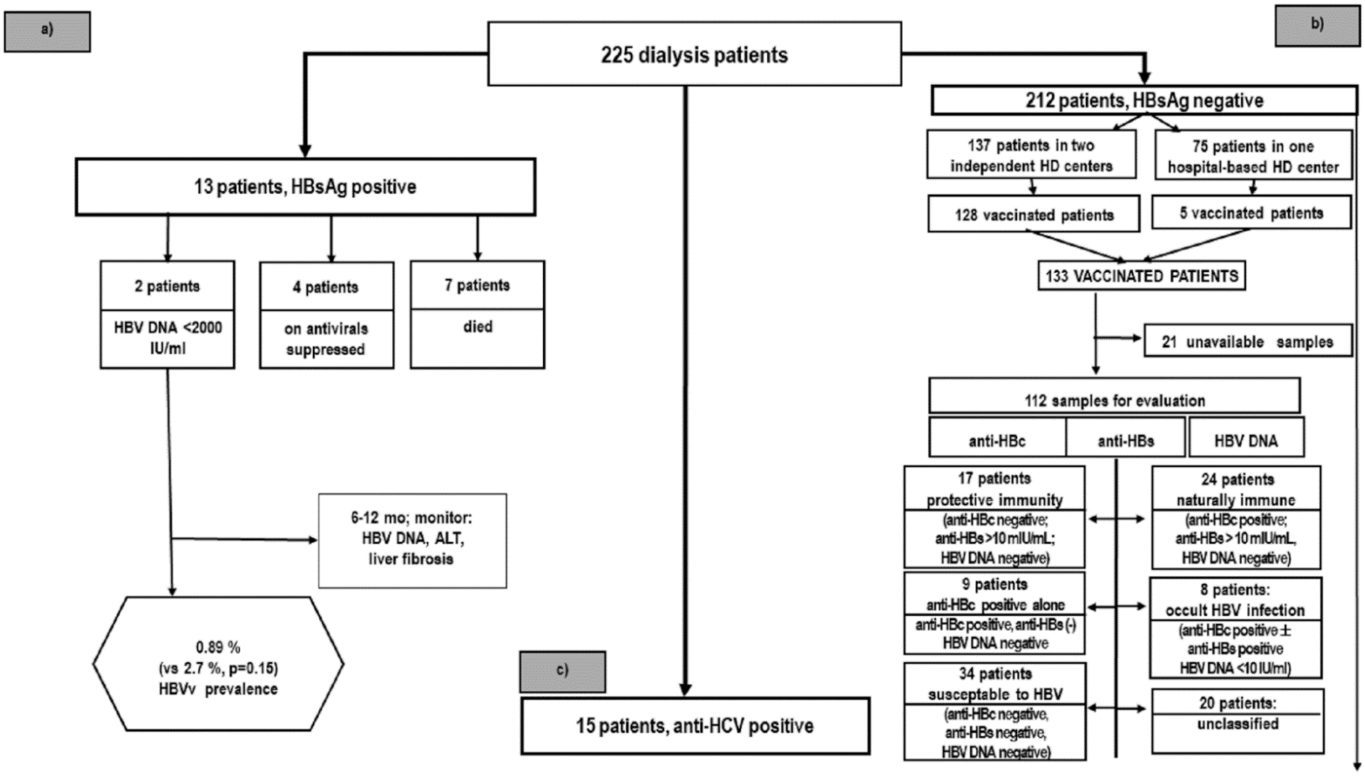
The 13 patients, infected with HBV, were further tested for the presence of HBeAg and anti-Hbe. HbeAg was negative and anti-Hbe was positive in all samples. Of these, seven patients died during the study, of which four had HBV DNA-positive results. Two succumbed before the initiation of antiviral therapy, while for the other two, this treatment was not indicated. Neither of them died of liver complications. (Figure 2a).

Of the remaining six patients, four were virologically suppressed via treatment with nucleos(t)ide analogs (n = 3, tenofovir disoproxil fumarate, n = 1, lamivudine). The other two cases were low-viremic (HBV DNA < 2000 IU/mL) and with normal alanine aminotransferase levels (ALT); therefore, they were not eligible for antiviral treatment [18]. These patients were tested for HBV DNA after 1 year, and the low viremia persisted. They accounted for a decreased HBV viremia (HBVv) prevalence (6/225 [2.7%] in 2020 versus 2/225 [0.89%] in 2022) (p = 0.15). HBV genotyping was successful in three of six HBV DNA-positive samples and the viral isolates were characterized as HBV genotype D.

HBV susceptibility

During the study, anti-HBs were tested only in HBsAg-negative patients treated in the two independent HD centers (n = 137). One hundred and twenty-eight were previously vaccinated with the HBV vaccine (128/137; 93.43%). Vaccination coverage was much higher than that in the hospital HD center, where 75 patients were eligible for vaccination, but only five received the HBV vaccine (5/75; 6.67%). Overall, HBV vaccination coverage was 62.74% (133/212). Out of the 133 vaccinees, 112 serum samples were available for further analysis of HBV susceptibility by anti-HBc, anti-HBs, and HBV DNA testing, the latter positive only in the cases of OBI (see below) (Figure 2b). Among these 112 cases, an anti-HBc - positive pattern alone (combination of undetectable anti-HBs in HBsAg-negative/anti-HBc–positive) was detected in nine patients (9/112; 8.04%). Surprisingly, 24 patients (24/112; 21.43 %) had acquired natural immunity to HBV (positive for both anti-HBs and anti-HBc). Despite the high vaccination coverage, only 17 participants (17/112; 15.18 %) had developed a protective serological response. Additionally, one-third of vaccinees (34/112; 30.36%) had remained

Figure 2. HBV infection in hemodialysis patients (n = 225). a) Hemodialysis patients with HBsAg -positive results (n = 13), b) Evaluation of HBV susceptibility in vaccinated hemodialysis patients (n = 112), c) § Refer to Figure 1.



HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; anti-HBc: hepatitis B core antibody; anti-HBs- hepatitis B surface antibody; ALT: aminotransferase; HBVv: HBV viremia.

susceptible to HBV infection (negative for anti-HBs and anti-HBc). Participants tested for only 1 or 2 of the 3 markers formed an unclassified subgroup of 20 (20/112; 17.86%).

HBV DNA was detected in eight cases, giving an OBI prevalence of 7.14 % (8/112). They presented seropositive OBI (n = 3 for anti-HBc and n = 5 for both anti-HBs and anti-HBc). Six of these 8 cases had HBV DNA tested a year later. In 4 of these 6 cases, both with positive anti-HBc/anti-HBs results, we did not detect HBV DNA. Still, two other patients with isolated anti-HBc had detectable HBV DNA (Table 2). No OBI case was anti-HCV positive. Noticeably, in three of the five HCV-viremic patients cured in 2018, OBI was diagnosed and pretreated with lamivudine. Three patients died at the end of the study of non-liver complications.

Discussions

To our knowledge, this study has assessed for the first time the characteristics of HCV and HBV infections among long-term HD patients in Bulgaria using highly sensitive molecular and serological methods. Our results have shown that though not statistically significant, a downward trend is noted for decreasing the prevalence of HBVv and HCVv infections among HD patients with a similar frequency but with different clinical significance. All HBV-infected cases on antiviral therapy were virologically suppressed. Furthermore, except for the two potentially curable HCV-viremic patients who refused treatment, all HCV-infected patients were treated with DAAs and cured. Worryingly, a significant proportion of those vaccinated did not have protective anti-HBs levels. Thus, they remained susceptible to infection or at risk of HBV reactivation in case of severe immunosuppression. Finally, we found an OBI prevalence of 7.14 %.

Viral hepatitis B and C prevalence is higher in HD patients than in the general population and varies between countries and from one HD unit to another HD unit within the same country [19,20]. In the most recent Dialysis Outcome and Practice Pattern Study (DOPPS) phase (2012-2015), the overall HCV prevalence among adult HD patients in 21 countries worldwide is 9.9% [21]. Regarding HBV alone, the pooled prevalence of HBV infection in HD patients is 7.32% [22]. Viral hepatitis infections are declining among HD patients worldwide. This decline reflects the reduced use of blood transfusions, screening of blood products for HCV and HBV, extensive use of HBV vaccination and antivirals for HBV infection, and implementation of infection control measures within HD centers [23]. In our country, HBV vaccination has been mandatory since 1992 for newborns. Immunization of HD patients who are HBsAg negative is highly recommended [14]. Regardless of that, immunization coverage is quite uneven among HD recipients. In our study, the overall coverage among HBsAg-negative patients was 62.74% (133/212).

Most studies of HCV infections, including the large-sized DOPPS, relied on widely used serological testing. However, the actual HCV prevalence might be overestimated. Though very rarely, some HD individuals might clear the virus spontaneously but continue to be anti-HCV positive [24]. Others, cured with DAAs, would remain anti-HCV positive with undetectable HCV RNA.

Epidemiological data on HCV infection in HD patients in Bulgaria are not up-to-date, while those on HCVv prevalence are scarce. One study alone discussed HCVv in HD patients before the introduction of DAAs [25].

The five patients, cured with DAAs before their enrollment in the study, tested HCV-RNA negative. We confirmed the durability of their SVR achieved several years ago. Noteworthy that, thanks to our study, another three HCV-viremic patients were cured with DAAs. The remaining two HCV-viremic patients refused DAAs and, thus, continue to be a threat of nosocomial transmission.

Table 2. Hemodialysis patients with occult HBV infection (n= 8)

HD start	Age	Gender	Anti-HBs	Anti-HBc	Anti-HCV	HBV DNA*	HBV DNA (one year later)	Outcome (2 years later)
2016	60	female	-	+	-	< 10	< 10	alive
2020	75	male	-	+	-	< 10	< 10	alive
2017	65	male	-	+	-	< 10	NT	alive
2016	64	female	+	+	-	< 10	NT	alive
2016	72	male	+	+	-	< 10	0	death
2014	68	male	+	+	-	< 10	0	alive
2018	81	female	+	+	-	< 10	0	death
2018	74	male	+	+	-	< 10	0	death

NT: not tested; * HBV DNA (IU/mL). anti-HBc: hepatitis B core antibody; anti-HBs: hepatitis B surface antibody; anti-HCV: hepatitis C antibody; HD: hemodialysis.

In our study, HCVv prevalence decreased, although not statistically significant (from 2.22% to 0.89%). Miyasaka *et al.* report similar results (0.79% HCVv) [26]. In kidney transplant recipients still lower HCVv prevalence of 0.08% is recorded [27]. Considering the shorter life expectancy in HD cases and less active HCV infections in the general population, we assumed DAAs as the main factor contributing to lower HCVv prevalence in our study. The advent of DAAs has dramatically changed the landscape of HCV therapy [28]. As stated by American and European guidelines, all patients with active HCV infection, defined as detectable HCV RNA in the blood, should be treated for HCV [16,29].

Kidney Disease Improving Global Outcomes (KDIGO) guideline recommends that all patients with chronic kidney diseases, on dialysis, and kidney transplant recipients with viremia be evaluated for DAAs [30].

As of 2017, DAAs have been reimbursed for viremic cases in Bulgaria, the year when our first five HD cases were treated and cured [31].

HCV infection does not confer protective immunity after viral clearance, nor does the achievement of SVR preclude the risk of reinfection. Therefore, in some high-risk populations such as MSM, HIV, IDUs, and HD patients, screening for HCV should be continued [30]. The absence of viremia in five HD cases cured with DAAs in our study for more than 6 years, ruled out the possibility of reinfection. Similarly, in a recently published paper, Liu *et al.* [32] concluded that HCV reinfection in HD patients achieving SVR with DAAs occurs infrequently and is comparable to that in the low-risk general population.

Of particular interest were the two anti-HCV-positive patients with numerous HCV RNA-negative results who had started HD treatment in their mid-twenties. The positive immunoblot results ruled out the possibility of a false-positive anti-HCV. We refrain from interpreting these HCV RNA negative results as an absence of replication, as intermittent or fluctuating HCV viremia is a well-known phenomenon in HD patients [33]. Therefore, we tested these cases repeatedly negative for HCV RNA and, in fact, almost excluded such a possibility. Importantly, one of these patients had undetectable HCV RNA in a previous study of ours [25]. We hypothesize that the patients had cleared their HCV infection earlier in their youth. Another plausible explanation could be the presence of occult HCV infection when HCV RNA is detectable only in the hepatocytes or peripheral blood mononuclear cells but absent in the blood. However, occult HCV is rare in HD patients, and detection methods are not readily available [34].

Finally, in all 210 anti-HCV-negative patients HCV RNA was undetectable. Thus, we excluded the possibility that some viremic individuals remained undiagnosed and untreated.

Special attention was paid to the two patients with low-level HBV viremia who did not meet treatment criteria. They accounted for a reduced HBV prevalence, also not statistically significant, probably due to the short life expectancy in HD patients. Still, given the fluctuating nature of HBV replication and liver inflammation, they should also be closely evaluated, at least annually, for HBV DNA and ALAT levels, to assess viral replication and candidacy for HBV treatment [35].

Many participants in our study had already received the HBV vaccine, but in very few of them, protective anti-HBs levels were detected. Moreover, in a third of the vaccinees, the immunological response was not even detectable. We cannot rule out the possibility that antibody concentration may have fallen below detectable levels years after vaccination. Considering the limited and short-lived effectiveness, a 4-dose schedule with a double-dose HBV vaccine is recommended. Post-vaccination anti-HBs testing should be assessed annually, and a single dose should be administered if anti-HBs fall to < 10 mIU/mL [36,37]. Some other patients exhibited natural immunity to HBV; therefore, we assumed that they had had previous exposure to HBV. Still, they were at risk of HBV reactivation in case of severe immunosuppression [38]. Also, nine patients displayed an anti-HBc-positive alone pattern. We supposed they had resolved HBV infection with spontaneous waning of anti-HBs over the years, as all were HBV DNA negative. However, they were also at risk of HBV reactivation in the case of chemotherapy or potent immunosuppressive therapy. Our findings highlight the need to update the HBV vaccination policy for HD patients in the country

Finally, eight vaccinees were diagnosed with seropositive OBI. In two of them, both with isolated anti-HBc, HBV DNA was still detectable a year later. Interestingly, as reported by Makvandi *et al.* [39], in most cases of OBI, as also in our study, the viral load was very low, about 20 IU/mL. The gold standard of OBI diagnosis is HBV DNA detection in the liver tissue, a specific but more restrictive definition [40]. Although easily tested, HBV DNA in the blood is usually present at low concentrations but may only be intermittently detected in individuals with OBI.

Therefore, testing of blood samples collected at more than one time- point and with highly sensitive assays is recommended [41]. In the eight OBI cases in our study, four tested negative for HBV DNA after one year. Indeed, without follow-up testing, we could not determine whether this result was due to the fluctuating nature of HBV DNA or whether the patients lost it.

Isolated anti-HBc, as detected in two OBI cases in our study, may often be considered a surrogate marker of the persistence of OBI and a considerable risk factor for OBI in HBsAg-negative subjects [42]. However, in a recent meta-analysis, Im *et al.* [43] stated that isolated anti-HBc should not be used as a sole marker for screening donated blood, even in resource-limited countries. On the other hand, even at low concentrations, HBV DNA can still be detectable for a long time with the ensuing consequences. HBV reactivation in the case of an immunosuppressive state leads to overt and even fulminant hepatitis or accelerates the progression of chronic HBV infection toward liver cirrhosis and hepatocellular carcinoma. Lastly, OBI can be transmitted through blood transfusion, stem cells, or organ transplants. These data are relevant to recognizing the HBsAg-negative persons at a higher risk of HBV reactivation before immunosuppressive therapy or with chronic liver diseases - of developing liver carcinoma [43].

Findings on OBI in Bulgaria are sparse. Damyanova *et al.* [44] reported 14 (17.72%) cases of OBI out of 79 patients with chronic liver dysfunction. Further studies are needed to evaluate the clinical significance of OBI in HD patients in the country.

Several limitations of our study should be addressed. Imaging investigations were not regularly performed before and after DAA treatment to estimate the level of liver fibrosis. Secondly, we could not follow up on all HBV- and HCV-infected patients due to the short survival rate of HD cases and COVID-19-related work demands. In addition, not all HBsAg-negative cases were tested for HBV DNA; therefore, the true prevalence of OBI might have been underestimated. Finally, biochemistry parameters, such as ALT levels, were not consistently studied.

Despite the above limitations, the application of sensitive molecular and serological methods among HD recipients has allowed us to assess HCV and HBV infections and patient management comprehensively.

Conclusions

The present study revealed a downward trend in the HBVv and HCVv prevalence among HD patients in Southern Bulgaria. It can be concluded that the overall risk of nosocomial transmission of HBV and HCV infection was significantly reduced. The reduction was achieved primarily by (i) suppression of viral replication in HBV-infected patients by nucleos(t)ide analogs, (ii) careful monitoring of HBV-infected patients in whom this treatment was not indicated, and (iii) achieving SVR in most patients with HCV-viremia. However, more than half of vaccinees remained susceptible to HBV. To further reduce this risk, HBV vaccination requires updating.

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Authors' Contributions

Study design and conceptualization: RK, AK, EG, data collection: RK, VR, microbiological investigations: EG, MV, Ch C, AS, TT, data analysis and interpretation: RK, AK, EG. Initial draft of the manuscript: RK, LNG, critical revision of the article: all authors, Final approval of the version submitted for publication: all authors.

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Conflict of interests

No conflict of interests is declared.

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