Monday 19 February 2024

Dear Editor of *World Journal of Hepatology*,

We would like to thank you and the reviewer for your positive feedback and the opportunity to revise our manuscript for publication in *World Journal of Hepatology*.

Our responses to the reviewer comments and specific comments from the Editor are detailed below, with the revised blue text in italics. As advised, we have also highlighted the revised/added content with yellow colour in the revised manuscript.

We hope that our amends are acceptable, and our manuscript is now suitable for publication.

We look forward to hearing from you in due course.

Yours sincerely,

Professor Liana Gheorghe

Reviewer comments

1. **Which tests are used for checking anti-HDV immunoglobulin G antibodies by enzyme-linked immunosorbent assays?**

These additional details have now been added to the Materials and Methods section, as follows:

All HBV-positive participants were tested for anti-HDV immunoglobulin G antibodies by enzyme-linked immunosorbent assays *(HDV antibody ELISA kit, Dia.Pro, Milan, Italy)*, following implementation of a policy agreement between *hepatologists and virologists at our gastroenterology centres.*

2. Which single and nested PCR tests (commercial?) are used for HDV RNA?

These additional details have now been added to the Materials and Methods section, as follows:

If the test result for anti-HDV antibodies was positive, subsequent reflex testing of HDV RNA was also performed by single and nested polymerase chain reaction amplifications of a highly conserved region of the HDV genome, using primers selected from genotype 1 of HDV *(RoboGene HDV RNA Quantification Kit 2.0, Roboscreen GmbH, Leipzig, Germany).*

3. All variables using in multivariate multiple regression analysis in materials and methods

All variables used in the multivariate multiple regression analysis are now mentioned in the Materials and Methods section. Please refer to the text below:

Using multivariate multiple regression analysis, variables identified as risk factors for HBV/HDV coinfection from the univariate multiple regression analysis were investigated. *These variables included sociodemographic factors (participant age, gender, residence [urban or rural] and education level [no or elementary school, high school, college/university]) and*

medical history (previous documented coronavirus disease 2019 [COVID-19] or comorbid diabetes mellitus). The HBV vaccination status of participants and their life partners was also included, as were the existence of any known family members positive for HBV/HCV/HDV (monoinfection or coinfection) and sexual contact with a partner positive for HBV/HCV/HDV (monoinfection or coinfection). In addition, exposure to healthcare procedures was considered; variables included were an occupational risk of exposure to blood products, history of blood transfusion, haemodialysis in antecedents (long-term or incidental owing to complications in an intensive care unit), any surgery before diagnosis (excluding dental surgery), at least one hospitalization before diagnosis and any dental surgery before diagnosis. Other risk factors included as variables were any history of severe accidents (work, traffic, domestic), record of accidents with blood-contaminated objects, history of injections at home or at an outpatient unit, imprisonment (current or previous), tattoos or any body piercing, injecting drug use, multiple sexual partners in the past three years, previous sexually transmitted diseases and history of abortions in improper conditions. All statistical tests were two-sided, and a *P* value of less than 0.05 was used to indicate statistical significance.

4. Is there hypothesis explained higher proportion of female HBV/HDV co-infected compare to HBV-monoinfection, take into account high imprisonment and injecting drug use in HBV/HDV co-infected compare to HBV-monoinfection?

There was no difference in the proportion of men or women with 'imprisonment' or 'injecting drug use' risk factors. Women had significantly higher rates of blood product transfusions and tattoos or body piercings compared with men. Further, unsafe abortion practices may have played a role. This has now been addressed in the Discussion section, as follows:

A higher proportion of women had HBV/HDV coinfection than HBV monoinfection in the current study. Abortion was restricted between 1966 and 1989 in Romania.[31] Unsafe abortion practices, particularly in settings where access to safe reproductive healthcare services was limited, may have posed a significant risk of viral transmission. Historical practices, policies and societal conditions may have shaped patterns of infection transmission and healthcare practices, leading to disparities between genders.

5. Provide also percentage of HBV viremia was less than 20 IU/mL in HBV-monoinfection

These additional details have now been added to the Results section, as follows:

HBV viremia was less than 20 IU/mL in *1.79% and* 27.9% of patients with *HBV monoinfection and* HBV/HDV coinfection at diagnosis*, respectively.*

6. Is there more data on combination therapy of pegylated interferon-α and nucleos(t)ide analogues in patients with HBV/HDV coinfection: the simultaneous "dual" strategy, sequential combination "add-on" strategy, and "switch" strategy?

These data were unfortunately not collected as part of the study.

7. How can you explain, that 12.5% of patients are vaccinated against HBV in HBV/HDV co-infected and 15.6 HBV-monoinfection groups?

Additional details have now been added to the Discussion section, as follows:

HBV vaccination was noted in 15.6% and 12.5% of people with HBV monoinfection and HBV/HDV coinfection, respectively. The effectiveness of the HBV vaccine can be reduced in people with certain risk factors, including older age, obesity or other chronic illnesses.[32] Some study participants may not have received all doses of the vaccine required for full protection.[33] The difference in HBV vaccination rates between these groups probably reflects a combination of factors related to healthcare access, provider practices, patient characteristics and the complex interplay between HBV and HDV infections.

8. Sexual contact with a partner positive for HBV/HCV/HDV means all three infections simultaneously (coinfections) or two or even one of mentioned?

This covers monoinfection or any combination of coinfection. This has now been addressed in the Materials and Methods section and in Table 2 as follows:

The HBV vaccination status of participants and their life partners was also included, as were the existence of any known family members positive for HBV/HCV/HDV ***(monoinfection or coinfection)*** *and sexual contact with a partner positive for HBV/HCV/HDV* ***(monoinfection or coinfection).***

9. Hepatocellular carcinoma diagnosis and management standardized (national guidelines) in Romania?

There are no national HCC guidelines in Romania; we are following the EASL and AASLD guidelines. These international guidelines are considered the standard of care for HCC in Romania.

Specific comments (from the Editor)

1. Please provide the Figures cited in the original manuscript in the form of PPT. All text can be edited, including A,B, arrows, etc. With respect to the reference to the Figure, please verify if it is an original image created for the manuscript, if not, please provide the source of the picture and the proof that the Figure has been authorized by the previous publisher or copyright owner to allow it to be redistributed. All legends require a general title and explanation for each figure. Such as A: ; B: ; C: .

The Figure has now been provided in editable PPT form. We confirm that this is an original figure created for the manuscript. A legend with general title and explanation for the figure has been included in the main manuscript text, as follows:

Figure 1 Prevalence of HBV monoinfection and HBV/HDV coinfection across different age groups. *A statistically significant difference in overall prevalence was identified between those with HBV monoinfection and HBV/HDV coinfection (P = 0.001). HBV: Hepatitis B virus; HDV: Hepatitis D virus.*

2. The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text (and directly before the References).

An Article Highlights sections has been added to the manuscript.

ARTICLE HIGHLIGHTS

Research background

Chronic hepatitis D virus (HDV) infection is associated with the most severe form of viral hepatitis. Certain regions, particularly Eastern European countries such as Romania, are characterized as 'endemic pockets' with hyperendemic levels of HDV infection. Given the emergence of dedicated antiviral therapeutics for HDV, an updated understanding of HDV epidemiology and clinical management is imperative. Although epidemiological data on hepatitis B virus (HBV) and HDV coinfection in Romania exist from previous studies, updated research is needed to accurately target high-risk populations for diagnosis and treatment.

Research motivation

Accurate data on HDV prevalence and health burden are sparse, hindering effective public health interventions. Additionally, the availability of novel antiviral therapies for HDV underscores the importance of revisiting the clinical management landscape. Despite recent advancements, treatment access issues persist, particularly in regions heavily affected by HDV. Addressing these gaps in knowledge is essential for developing targeted strategies to combat HDV infection effectively.

Research objectives

To investigate the epidemiology, natural history, risk factors and clinical management of HBV/HDV coinfection in patients in Romania.

Research methods

This prospective cohort study was conducted in six tertiary gastroenterology and hepatology referral centres in Romania. Eligible participants were adults (≥ 18 years) who tested positive for HBV. Data regarding patient demographics, disease stage, previous therapy use and risk factors for HBV and HDV infection were collected via patient questionnaires and medical charts.

Research results

The hospital-based prevalence of HBV infection was 3.8% (95% confidence interval [CI]: 1.8-5.8). Overall, 963 individuals were HBV-positive and were enrolled in the study; the prevalence of HBV/HDV coinfection was 33.1% (95% CI: 31.2-35.1). Over 90% of patients with HBV/HDV coinfection were treated, with 42.5% receiving pegylated interferon-α therapy, 36.4% receiving nucleos(t)ide analogues and 49.6% receiving combination therapy. Multivariate multiple regression analysis identified several independent risk factors for HDV infection: female gender ($P = 0.0006$), current or previous imprisonment ($P < 0.0001$), older age at diagnosis ($P = 0.01$) and sexual contact with a partner positive for HBV/HCV/HDV (monoinfection or coinfection, $P = 0.0003$).

Research conclusions

The present study emphasizes the need for systematic screening for HDV infection, subsequent reflex testing of HDV RNA and collaborative initiatives for controlling, treating and preventing HBV and HDV infection.

Research perspectives

Further investigation into the geographic variability of HBV and HDV epidemiology, including prevalence, distribution patterns and emerging trends worldwide would provide valuable

insights into regional disparities and the effectiveness of public health interventions. Evaluating the effectiveness and cost-effectiveness of novel screening strategies, including universal screening, double reflex testing and point-of-care testing will allow us to optimize early detection of HBV and HDV infection, particularly in resource-limited settings and high-risk populations. Continued research into novel therapeutic agents may improve clinical outcomes for affected individuals.

3. Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

We have no grant application or funding agency for this study.

4. Please provide the primary version (PDF) of the Institutional Review Board's official approval, prepared in the official language of the authors' country.

Requested document has been uploaded.

5. Please provide the Clinical trial registration statement.

This is not applicable for this manuscript, because this manuscript does not report results from a clinical trial. The current manuscript describes the results of an observational study. As such, it falls into the category of "Case Control study, Observational study, Retrospective Cohort study". We have included all documents listed in your guidelines (https://www.wjgnet.com/bpg/GerInfo/287) as required for this type of study.

6. Please provide the primary version (PDF) of the Informed Consent Form that has been signed by all subjects and investigators of the study, prepared in the official language of the authors' country.

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7. Please provide the CONSORT 2010 statement.

This is not applicable for this manuscript, because this manuscript does not report results from a clinical trial. The current manuscript describes the results of an observational study. As such, it falls into the category of "Case Control study, Observational study, Retrospective Cohort study". We have included all documents listed in your guidelines (https://www.wjgnet.com/bpg/GerInfo/287) as required for this type of study, including a STROBE statement. We have included some additional text in the study limitations paragraph of the Discussion section to ensure we meet the STROBE guidelines. We have also stated on the title page of the manuscript that the guidelines of the STROBE Statement have been adopted, as per the guidelines.

8. Please provide the Biostatistics Review Certificate.

Requested document has been uploaded.