Format for ANSWERING REVIEWERS

April 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9795-review.doc).

Title: Occult Hepatitis B Virus Infection Among Mexican HIV-1-Infected Patients
Author: Ma. Teresa Alvarez-Muñoz, Angelica Maldonado-Rodriguez, Othon Rojas-Montes, Rocio Torres-Ibarra, Fernanda Gutierrez-Escolano, Guillermo Vazquez-Rosales, Alejandro Gomez, Onofre Muñoz, Javier Torres, Rosalia Lira.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9795

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated and edited, comments have been included in the file.

2 Revision has been made according to the suggestions of the reviewers.

(1) *COMMENT: This study fails to include a control group (e.g. HIV/HCV coinfected patients; non-viral chronic hepatitis) useful to compare the prevalence of occult hepatitis B. I am puzzled by the very high prevalence of occult hepatitis B in this series, which raises the possibility of false-positive results.*

R. We appreciate your comments. First, in our study we could not include the control group you suggested because we did not count with HIV/HCV coinfected patients, neither with non-viral chronic hepatitis patients.
To avoid the false positive results we were very careful in the use of separate areas, to prevent sample cross-contamination and the samples were run by duplicate. Positive and negative controls were included in each run for the different assays. We also confirmed the results with real time PCR assays, negative controls were included in every run, and the samples were run by duplicate in two separate experiments. The core sequences that we could obtain showed some differences in the nucleotide sequences (Fig 1S) as it is shown in the phylogenetic tree built in the Mega program.

Moreover, metabolic factors, such as steatosis have not been taken into consideration

Because this was a cross-sectional study, liver and clinical hepatic characteristics were not determined in our study, resulting impossible for us to analyze the impact of metabolic factors in the HBVO co infection. However, it would be very interesting to evaluate in future studies with HIV patients, the level of steatosis and the possible association with OHBI. Several studies have addressed the prevalence of liver steatosis in HIV-infected patients, and it has been reported high prevalence of this condition in HIV patients HAART experienced (Virol J. 2013 Aug 14;10:261). It has also been reported that patients under antiretroviral treatment are in risk to develop severe liver damage that progresses to fibrosis, cirrhosis and even terminal liver disease. The article you suggested to discuss Loria P et al, is a complete review the correlation of hepatic steatosis and the most common chronic liver diseases due to

different etiologies, it would be interesting to find out in the future, if the steatosis is a metabolic factor associated with Occult hepatitis B co-infection. The other references you suggested were also reviewed.

A specific paragraph on the several limitations of this study needs to be added. Based on the many limitations of the study design.

Our study had some limitations. The specific paragraph has been included in the discussion section of the manuscript. The limitations of our study are:

a) The sample size was relatively small and it was a single time point testing without any follow-up; B) this was a cross-sectional study where liver and clinical hepatic characteristics were not determined. However, the data obtained in here is very important and valuable to suggest the need to determine the co infection especially in HBsAg negative patients.

In our country, especially in security social Institutions, we did not count with enough resources to perform all the serological markers for HBV infection, the problem is that in negative HBsAg patients, there is no other evidence about OHBI/HIV coinfection, for this reason, we strongly recommend that routine testing for patients newly diagnosed with HIV/HBsAg negative should include tests for HBV-DNA in Mexico.

(2) *The authors should address any issues relating to possible cohort bias.*

R. We appreciate your comments. We consider that possible cohort bias are: a) the sample size was relatively small and it was a single time point testing without any follow-up; b) since it was a cross-sectional study, not liver and clinical hepatic characteristics were determined in our study.

Finally, the study population depended on the number of volunteers we could include for the study. Generally, the patients included in this study are long-term of controlled HIV-VL and long-term of HAART treatment.

The patient selection was random, the Physician in charge of the medical follow-up invited the HIV patients, and 13 patients from the Hospital No. 72 were invited if they presented any HBV serological marker, for example antiHBcAg that is frequent in the Mexican HIV population.

To emphasize the size of the study, the conclusion should again mention the number of patients involved

In the conclusion the number of patients involved in the study has been included.

(3) *Sample size is too small to determine the frequency of OHBI in HIV-1+/HBsAg- patients. They should include more patients*.

Response: This was a transversal study where we utilized a relatively small sample size for detection of OHBI in a group of HIV-1+/HBsAg- patients. We invited HIV-infected patients to participate as volunteers (signed informed consent). We could include 49 patients during the period of the financial support. We are unable to increase the patient number, however our data was carefully performed and analyzed and we consider that the frequency of occult hepatitis B infection in HIV-1+/HBsAg- patients found in our study, suggest that HIV/OHBI coinfected patients remain undiagnosed, if only conventional serological markers for HBV are used. The contribution of this initial study will allow us to apply for more financial resources in order to explore in the future a bigger HIV-1 population and also obtain more statistical power results. We suggest that the first screening with the in-house nested PCR assay will reduce the cost in the diagnosis of these patients.

2. *They should show the frequency of OHBI in HIV-1-/HBsAg- population in the table 2.*

The frequency of OHBI in HIV-1-/HBsAg- population has been included in table 2.

.....

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Rosalia Lira, Ph.D.
Laboratory of Virology
UIMEIP, IMSS
Mexico City
ZP 06720-Mexico
Phone +52-55-56276900 ext 22407
E-mail: rolica36@yahoo.com