Author's response to reviews

Title: Hepatitis B virus: Molecular genotypes and HBeAg serological status among Brazilian patients.

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Author's response to reviews: see over
We are sending the response to reviewers about the manuscript “Hepatitis B virus: Molecular genotypes and HBeAg serological status among Brazilian patients” MS: 1846870675262152.

Thank you for giving us some extra time.

Best regards

Neiva Sellan Lopes Gonçales
Response to reviewers

Reviewer: Yu-Ching C Lan

Reviewer's report:
* Major Compulsory Revisions

The main issues in this manuscript provided the HBV patients’ clinical features in a hospital at Campinas city, Brazil. Although the results were similar with other papers from Brazil, this manuscript would provide local descriptive epidemiology information which is important for public health work by improve following information.

1. Does the study population in this research different with other place? The geographic or nationwide comparison of demographic data (gender, age, race, risk factor) in Table 1 would help to understand the patients' characteristics in Southeast Brazil.

R: Yes. Mainly in the Amazon area where Amerindian descendants were the major population and because of this genotype F is the most prevalent. In the Northeast of Brazil, especially Bahia state, there are African descendants in a great number than other races. Because of this it is necessary to consider that genotype Aa is more common than others. Brazil is a country that has mixed population and differs from area to area according immigration in the past in each area.

I did not include other regions of Brazil in Table 1 because our study data were only based on patients who live in our area and attend our hospital. There are references in others parts of this paper to other regions already studied. Please see the last paragraph in the background section and also the first paragraph of the discussion.

2. The researcher used the sequence comparison method to identify the HBV genotype but lack the precise definition (for example: the homology with the specific genotype reference sequence; with or without mutation; phylogenetic analysis).

R: The analyses of the genotypes and viral characteristics were carried out using the Bioedit program version 5.0.6. Sequences were then submitted to Blast program in order to check their similarity to other HBV strains (accept concordance between query and subject up to 90%) deposited in the Genbank. The genotypes were assigned to known groups using phylogenetics inference with known genotypes corresponding to eight HBV genotypes identified in humans and deposited in the Genbank: genotype A (X75666, EF690536; subgenotype A1= AF418672, subgenotype A2= AB64314; subgenotype A3 (Acmr) = AB194951), B (X75660 ayw1; D00330 adw2; AB191369): C (D12980 adr; EU678472; AB24113.1); D (AB090270; X75668 ayw3; X59795 ayw2) E (X75657; AP007262; X75664) F (X75658; X69798 adw4; AY179735) G (AP007264; AB191378) H (AB191383; AB298362) and Genotype G/C recombinant (DQ078791).
3. The result for Table 3 had smaller a sample size in each genotype. The statistic power would be stronger if all 139 cases were processed for genotyping. We agree with your consideration but the number of patients in Table 3 was smaller than the whole studied group because only those who had the clinical and laboratorial criteria to be submitted for liver biopsy were included. The biopsy is a procedure indicated by clinicians according to specific criteria. In our study only 65 patients had all the criteria for submission to a liver biopsy.

4. The statistical analysis also needs more definite description. (Some of the statistical tests in Table 2 need chi-square.) The chi-square was used but no difference was encountered during this analysis of data and the p value was maintained.

* Minor Essential Revisions
1. Methods, the Patients paragraph second line
mean age 42+14.5 should be 42±14.5
R: According to your observation, we corrected the mean age.

Reviewer: Chi-Ju Chen
Reviewer’s report:
Summary:
Tonetto et al. present a paper on HBV genotypes and HBeAg serological status among 139 people from southeast Brazil. Viral load, ALT level and liver fibrosis stage were compared among different genotypes and HBeAg status.

Major comments:
1. In the Background section, authors should review more published studies regarding HBV genotypes in Brazil. According your suggestion others relevant studies published in Brazil were revised and were included in this manuscript.

2. HBeAg-positive often means more active HBV replication in a patient. Among 70 HBV DNA positive samples, only 17 were HBeAg-positive while 53 were HBeAg-negative. How is this explained?
R: Your question was based on data about HBV-DNA quantification. In reality, all samples studied (139) were HBV-DNA positive and were genotyped and of the total 97 (70%) were HBeAg negative and 42 (30%) HBeAg positive (Table1). Therefore only 70 samples were possible quantify at the moment of study. Of these 53 were HBeAg negative and only 17 were HBeAg positive. In table 2 the difference between the HBeAg positive and HBeAg negative group can be observed.
I hope this explanation clears up your question. We will add this information to the manuscript for clarification.
3. Table 3 was not referred in the text. The content of Table 3 should be better described in the text. Is presence of HBeAg a significant factor in liver fibrogenesis?
R: We observed that this was excluded from Table 3 in the manuscript. It will be included in the correct position.

"Among the 65 patients who were submitted to the liver biopsies (Table 3), a mean stage of fibrosis of 2.4 was determined for genotype A, 1 for the genotype C, 2 for genotype D and 3 for genotype F (P = 0.1148). On the other hand, the mean stage of fibrosis in genotype A patients (2.8) was found to be significantly higher than the mean stage of fibrosis in genotype D patients (2.0) (P= 0.0179). Therefore, there was no significant difference between the grades of fibrosis when the different genotypes were compared, according to the presence or absence of HBeAg (Table 3)".

4. Upon randomly checking, wrongly cited references were found. For example, in Discussion, reference 19 and 28 are wrongly cited. Typos were also found. All the citation must be checked out. Reference 29 (Kao et al.) is a study from Taiwan not Japan.
This has been corrected.

Minor comments:
1. In the Methods, primer positions in the HBV genome should be mentioned in “HBV DNA detection”. PCR amplification of a 417-bp fragment, which was used for sequence, should be better described.
R: Your observation was added in the methods section of the manuscript.

"The selected region for amplification also covers the amino acid loop corresponding to a,d/y and w/r specificities and mutations related to HB Ig, anti-HBs monoclonal antibody and vaccine resistance. Selected primers were as follows: HBS1F 5′-GAG TCT AGA CTC GTG GTG GAC TTC-3′ and HBS1R 5′-AAA TKG CAC TAG TAA ACT GAG CCA-3′ and inner HBS2F 5′-CGT GGT GGA CTT CTC TCA ATT TTC-3′ and HBS2R 5′-GCC ARG AGA AAC GGR CTG AGG CCC-3′). The primer positions in the HBV genome (strains HBV ADW; Genbank accession number V00866) were as follows: HBS1F (positions 244 to 267) HBS2F (positions 255 to 278) HBS2R (positions 648 to 671) and HBS1R (positions 668 to 691) [20]”.

"The products of nested PCR were subjected to cycle sequencing reactions, as described previously [26] by using the second-round primers and the ABI Prism Bigdye Terminator Ready Reaction kit (Applied Biosystems, Foster City, CA). After purification samples were denatured and directly sequenced by automated cycle sequencing (ABI 377, Applied Biosystems, Foster City, CA). Revision of the nucleotide sequences was carried out using the BioEdit program version 5.0.6 [27]. Sequences were then submitted to the Blast program in order to check their similarity to other HBV strains deposited in the GenBank. The results of sequencing were consistent with those of HBV isolates obtained from GenBank, corresponding to the known HBV genotypes".
2. In the Results, numbers of patients were wrong in the text. For examples: One hundred and seven (84.2%) of them were Caucasian descendants should be 117. 96 (70%) of patents were HBeAg negative should be 97 (70%).
R: We corrected the number, according to your observation.

3. In Table 2, “HBeAg+ve or –ve” and “ALT<1.3 LSN” should be explained. Criteria for inactive carrier/chronic hepatitis should be clearly defined (HBsAg+, HBV DNA+ or -, ALT level). Range of HBV-DNA should be indicated.
4. In the fifth paragraph of the Discussion, “In other studies, considerable differences …varing from 52.5% to 63.3%”, the meaning is not clear. Authors also comment on the reason for 70% HBeAg-negative was due to the long infection. What is the base on this comment?
R: According to your observation we corrected the fifth paragraph of the discussion.

“…This demonstrates the high percentage of cases of HBV patients that were negative for HBeAg in our population. In other studies, with different populations considerable differences also were observed between the percentages of HBeAg-positive and HBeAg-negative patients. These authors encountered a higher prevalence of HBeAg-negative patients, varying from 52.5% to 63.3% [33-35]. Similar frequencies were also observed in another study in Brazil, in which 58% of the HBV patients were HBeAg negative [36]. About 30% of our patients were HBeAg positive and 70% were HBeAg negative. Probably the majority of the HBV positive patients in our region had been infected for a long time and had developed mutations in the pre-core region (HBeAg negative/anti-HBeAg positive).”

5. The fibrosis stage was significantly greater among HBeAg negative patients infected by genotype A. The authors commented that this is due to 18.3% of African descendents among their genotype A infected patients. This should be further elaborated
R: According to your comment about the data of Table 3. There is no significant difference between the stage of fibrosis in the HBeAg–positive patients and HBeAg-negative patients. There are only differences in the stage of fibrosis when we compared the data of genotypes A and D (Table 3)