

Format for ANSWERING REVIEWERS

June 17, 2014

Dear Editor,

Please find enclosed our edited manuscript in Word format (file name: 11186-review.doc).

Title: Mutations of Pre-core and BCP Before and After HBe Antigen Seroconversion

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 11186

The manuscript has been improved according to the suggestions of the Reviewers as follows:

1 Revisions have been made according to the advice of the Reviewers:

(1) *reviewer 01804246: This is an interesting paper well presented. There is need only for minor language corrections.*

Our manuscript has been polished by a professional English language editing company.

(2) *reviewer 00465536: The main limitation of this work, as indicated by authors, is the lack of entire genomic sequences to better investigate the mechanisms of "e" system seroconversion.*

We have since added entire sequence data and discussed them in the revised manuscript (page 7, lines 8-16, page 10, lines 6-10, page 11, lines 4-6).

Abstract, methods section: The sentence "...were analyzed before and after seroconversion in chronic hepatitis B patients who were persistently HBeAg positive (n=18), experienced HBeAg seroconversion (n=25), or were persistently anti-HBe positive (n=43)." is not clear since only 25 patients experienced seroconversion.

We appreciate the Reviewer's comment and have reworded the sentence (page 2, lines 7-9).

Introduction: short and well presented.

Thank you very much.

Material and methods 1/ Patients, second paragraph: it's better to put (for a better clarity) Table 1 (and corresponding text) in a 1st paragraph in the results section.

We have modified the paper according to the Reviewer's suggestion (page 8, lines 2-6).

2/ Determination of pre-C and BCP mutations, second and third paragraphs: the same, it's better to transfer in the results section the frequencies of mutations that the authors obtained (with 154 and 143 samples for pre-C and BCP determination, respectively).

We have adjusted the paper according to the Reviewer's suggestion (page 8, lines 8-11, page 8, lines 23-27).

Results 1/ Changes in pre-C and BCP mutations: The figures (1 and 2) are readable but the authors should try histogram bars (showing modifications in frequencies between start and end



of follow-up) to present their data in a more attractive way. Figure 3 is nice.

Thank you for your comments. We have changed figures 1 and 2 to those using histogram bars.

Discussion: The 1st paragraph of the discussion should be deleted.

We have deleted the first paragraph of the discussion.

- (3) *reviewer 00030389: Major comments. #1. There is significant difference, while the authors described them as tendency. Please described the findings correctly. Please discriminate significant differences from differences in tendency. Please show p values in the manuscript.*

We appreciate the Reviewer's comments. We have added P values in the manuscript and properly described significant findings as such.

#2. Introduction, line 25. Do the authors think that HBeAg seroconversion was not associated with either mutation? In the present study, they showed that HBeAg seroconversion was associated with pre-C mutation. In the previous study, did they actually observe the opposite results.

We apologize for the misleading statement. In the previous study, HBeAg seroconversion was not associated with either mutation in patients with inactive replication. We have reworded this sentence (page 4, lines 26-7).

#3. They selected the patients without antiviral therapy during the study period of several years. Thus they selected those who did not need antiviral therapy and had normal ALT levels. I worry that the bias of selection may cause the wrong results. Please discuss this problem in discussion.

We appreciate the Reviewer's concern. All patients were enrolled between 1985 and 2000. At this time, antiviral therapy had yet not been approved in Japan. Hence, we believe that there is no selection bias (page 5, line 7).

#4. Page 9, lines 15-17. Kawabe N, et al. reported that the loss of HBeAg without precore mutation results in lower HBV DNA levels and ALT levels in chronic hepatitis B virus infection (Journal of gastroenterology. 2009;44: 751-6). Please refer this report and discuss on it.

Thank you for your suggestion. We cited this report as new reference 28 and discussed it (page 11, lines 17-20).

Minor comments.#1. M&M. Determination of pre-C and BCP mutations "since the rate steadily increased to 100% once it surpassed that value". What does this sentence mean?

We have reworded this sentence according to its reference (page 6, lines 28-9).

#2. P6, line 2. "Of the 143 samples with determined BCP mutation types, 34 (24%) were wild, 11 (8%) were mixed, and 98 (69%) were mutant types". This sentence should be in the results.

We have moved this information as suggested (page 8, lines 23-7).

#3. "anti-HBe status subjects". "positive" should be inserted.

We have inserted the word "positive" throughout the manuscript, as suggested.

- (4) *reviewer 00742524*

Since the sample size is small, the full-length HBV sequence should be determined to find out whether there might be additional mutations.

Thank you for this comment. We have added entire sequence data and discussed them (page 7, lines 8-16, page 10, lines 6-10, page 11, lines 4-6).

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

Sincerely yours,

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