November 1, 2015

Dear Dr. Qi,

My co-authors and I are grateful for the editors' consideration of our manuscript and the reviewers' comments. Below are our responses to the editors' and reviewers' comments.

Editors' comments:

1. Regarding language certificate, English is the native language for the majority of the authors
2. In regards to adding a table or a figure, we do not feel that this would enhance the points of our paper
3. A running title has been included in the manuscript
4. We have moved the institutional board review statement, informed consent statement and conflict-of-interest statement to the appropriate area in the manuscript as required.
5. Please note that we have changed the corresponding author to Jennifer Leong, MD from Mindie Nguyen, MD by mutual consent.
6. The core tip has now been included after the abstract
7. The authors' abbreviated names and the manuscript title have been included after the core tip
8. In the main text, a typo was noted for the section title "Case Reports" and has now been corrected
9. The abbreviations have been moved to the end of the manuscript as per the guidelines
10. The references have been reformatted
11. The comments template has been completed

Reviewers' comments:

Reviewer #3020777:

No comments.

Reviewer #2462014:

Leong et al. reported two cases with chronically infected HBV featured by coexistence of HBsAg and HBsAb. According to the disease status, patient's serology and family medical history, as well as some literature reviews, there comes to an assumption that HBsAg escape mutations may contribute to this phenomenon. The two case reports are interesting containing some important information for HBV clinical management, but the crucial information for supporting this assumption doesn't have a clue in the manuscript. What HBsAg mutations exist if you analyze the HBV full genome derived from these two patients? If they do exist, how do these variants correlate with the decision to initiate early antiviral therapy and the risk of transmission? In case 1, what benefits do you bring when the pregnant mother received the antiviral treatment after 32 weeks gestation?

We appreciate the reviewer's comments. We agree and would have very much liked to have been able to analyze the patients for specific HBsAg mutations. However, this is unfortunately not available in routine practice, and therefore we were unable to obtain this information. Based on the clinical presentation and historical data, we felt strongly that the presence of HBsAg mutations was the most likely explanation for these clinical presentations. We feel that based on published reports of transmission of these mutated viruses in vaccinated individuals, the decision to initiate early antiviral therapy should be made on an individual basis, with risks and benefits being fully disclosed to the patient. In the scenario of case 1, although it is well documented that hepatitis B immunoglobulin and hepatitis B vaccination can decrease the risk of vertical transmission significantly, in the presence of HBsAg mutations, both interventions would be rendered ineffective and the risk of vertical transmission is still present particularly in the situation of a high viral load.

Reviewer #12216

J Leong et al describe two persistent HBV infection cases with low replication featured by the concurrent presence of HBsAg and HBsAb. They assume that this phenomenon is due to HBsAg mutation at the "a" region favouring the escape from the natural HBsAb. The first case is an

eAg+ chronic infection that is weird because of the low replicative status that is not common in e+ patients. The second case is an antiHBe+ patient with low replication and F0-F1 liver fibrosis that would be considered as an inactive carrier, but he was treated because of the possibility of having been infected by an HBsAg mutant virus due to the coexistence of HBsAg and HBsAb in patient's serology. The main caveat of both case-reports is that authors do not demonstrate the presence of the assumed mutations. I think they should sequence the HBV genome searching for mutations at the HBs Ag region. In fact, in the recent literature, cases with HBsAg and HBsAb coexistence have been described without HBsAg mutations (BMC Gastroenterol. 2014 May 17;14:94. doi: 10.1186/1471-230X-14-94). Authors should also show the HBsAb level, since non-protective HBsAb level could be seen in low replicative patients, in a previous status in inactive carriers before reaching natural immunity.

We recognize that a weakness in our paper is the inability to detect the HBsAg mutations, but we feel that it is because of this unavailability that physicians should maintain a level of clinical suspicion when encountering such scenarios. We hope that our experience may help other physicians when encountering such difficult clinical scenarios. Unfortunately, testing for the mutation was not available at the time. Quantitative anti-HBs levels were also not done and therefore no data is available for this.

In regards to the paper mentioned, we read this with great interest. This was the case report of a 59 year-old Italian male who had never been vaccinated for HBV, and did not develop anti-HBs until he was treated with interferon. Our cases presented with different scenarios. In case 1, the patient had been vaccinated as a child, yet despite development of anti-HBs, she developed HBV infection, likely through horizontal or vertical transmission as both her mother and sister have HBV infection as well, which supports the likelihood of the presence of a surface antigen escape mutation. In case 2, the patient's wife contracted HBV despite previously receiving HBV vaccination and documented presence of anti-HBs. We do not feel that all patients with coexistent HBsAg and anti-HBs should be treated based on these serologies alone, but that the entire clinical presentation must be taken into account and should be done so on an individualized basis.

Reviewer #12386

Leong et al. reported that 2 cases with HBsAg escape mutations. These cases seem precious cases.

1. If possible, please indicate the status of HBsAg and anti-HBs of her wife of case 2.

2. Please add "Tenofovir may be useful for the prevention of transmission of HBV with HBsAg escape mutations", and discuss more about these

Regarding comment 1, since the patient's wife is not our patient, we do not have access to her medical records. However, she is a nurse and clearly stated she was found to have active infection with positive HBsAg where she was previously without infection and was able to donate blood almost yearly until this discovery. She also stated she has undergone vaccination with protective immunity as required by her employment.

In regards to comment 2, we mention in the last paragraph of our discussion that initiating therapy may help reduce the risk of transmission – we have now changed this sentence to: "In situations where the risk of sexual, vertical, or horizontal transmission, patients and their family members should be counseled carefully, and consideration should be given towards having a lower threshold to initiating *potent antiviral* therapy in these individuals in order to reduce the risk of transmission."

We hope the above responses are satisfactory and thank you once again for considering our manuscript for publication in *World Journal of Clinical Cases*.

Sincerely,

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