ANSWERING REVIEWERS

15 of September 2012
Dear Editor,

Please find enclosed the edited manuscript in Word format (file name wjh-2012-165-review.doc).

Title: Differential expression of hepatic apurinic/apyrimidinic endonuclease 1, a DNA repair enzyme, in chronic hepatitis

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Manuscript No: wjh-2012-165-review

The manuscript has been improved according to the suggestions of reviewers:
1. Format has been updated
2. Revision has been made according to the suggestions of the reviewer
(1) In Materials and methods, Patients.
"4" normal liver tissue samples were obtained.....should be "Four" normal liver tissue samples were obtained.....
→I corrected it according to the suggestion.
(2) In Materials and methods, Patients.
All patients gave informed consent for liver biopsy. This does not make sense.
All patients received informed consent.....
→I corrected it according to the suggestion.
(3) In Materials and methods, Patients. "The study was performed in accordance with the Helsinki Declaration." Please add references or years.
→I added the years in the manuscript.
(4) In Materials and methods, Liver biopsies. "Histological characteristics of chronic viral hepatitis and AIH were evaluated using the standard criteria proposed by Desmet." Please add references.
→I added the references in the manuscript.
(5) In Materials and methods, Liver biopsies. "PBC liver specimens were staged according to the proposal of Ludwig." Please add references.
→I added the references in the manuscript.
(6) In Materials and methods, Liver biopsies.
"A portion of each sample from 69 patients (30 chronic hepatitis C, 27 chronic hepatitis B, 6 autoimmune hepatitis, 6 primary biliary cirrhosis, 4 normal livers)". The reviewer understands what the authors would like to show. However, as in "Materials and methods, Patients, The inclusion of 73 patients in this study," 69 should be 73.
→I corrected it according to the suggestion as described in the revised text.
(7) In discussion. "In this case, we were not able to identify the correlation with APE-1 as HBX (not shown), but, from a past report, as for APE-1, there may be carcinogenesis and connection." The grammar should be rechecked.
→I rechecked and improved it according to the suggestion.

(8) In this study, there was not a correlation of iron-related serum factor and expression of APE-1 protein. In other words there were not the data indicating the association between iron and oxidative stress. Have the authors examined the iron deposition in hepatocyte and compared the relationship?
→I would like you to reconsider this point because we have made extensive change in the discussion. I don't think that the data and discussion regarding iron parameters are neccessary in this revised paper considering the context in the discussion.
(9) APE-1 protein and mRNA expression showed a reduction in HBV patients compared with HCV patients. Also 8-OHdG expression showed a reduction in HBV patients. In the introduction, the authors state that ROS mediate and enhance APE-1 expression and activity, while APE1 controls the intracellular ROS production by negatively regulating the activity of the ROS-related guanosine triphosphate hydrolase (GTPase). Taken into "ROS enhance APE-1 expression and activity", Less oxidative stress causes less expression of 8-OHdG and thereby, less expression of APE-1 in HBV than others. Have the authors examined the ROS or the levels of oxidative stress?
→It is hard to measure ROS levels in the frozen samples. 8-OHdG is one of DNA modified ROS. We examined intracellular status of oxidative stress using immunohistochemical analysis for 8-OHdG.
(10) The conclusion that patients with chronic hepatitis B virus (HBV) infection, not chronic hepatitis C virus (HCV) infection, showed reduced expression of hepatic APE-1 and 8-OHdG compared with normal livers and the other chronic liver disease. This is understandable as a phenomenon occurring in these chronic liver diseases. Hepatic APE-1 and 8-OHdG expressions may undergo differential regulation in chronic HBV and HCV infection. Downregulation of hepatic these expressions may contribute to the development of hepatocellular carcinoma (HCC) in chronic HBV infection. This might be one of the mechanisms involved. But how about in the case of HCV which also causes HCC? If the oxidative stress is less in HBV than others, APE-1 and 8-OHdG might show less increment. How have the authors concluded the causal-relationships between ROS, APE-1, 8-OHdG, and carcinogenesis?
→In our original version, we showed that patients with chronic hepatitis B virus (HBV) infection reduced expression of hepatic APE-1 and 8-OHdG compared with normal livers. It does not make sense, so we tried to do immunohistochemistry for 8-OHdG in normal livers again. We got the data as described in the text and figure. And we have made some changes in the revised text and figure. The revised text includes some discussion regarding the causal-relationship between APE-1 and carcinogenesis.

3. References and typesetting were corrected
Thank you again for publishing our manuscript in the *World Journal of Hepatorology*.
Sincerely yours,

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