

ANSWERING REVIEWERS



March 18, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16829-Edited_Track_Changed.doc).

Title: Changes in gene expression in liver tissue from patients with fulminant hepatitis E

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Name of Journal: *World Journal of Gastroenterology*

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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 (comments are shown in italics, and responses in Roman font).

- (1) *This paper offers interesting data regarding the pathogenesis of fulminant hepatitis E, in order to better characterise the immune-mediated killing of infected cells. It is well written and results are convincing. It would be of interest to explain why the authors included a control group with fulminant hepatitis B and not other virus (for example HAV, which, indeed, share more of the pathogenesis mechanisms of infection with HEV than HBV). If available, I would also provide the viremia (HBV DNA and HEV RNA values) of patients in both groups (fulminant B and E hepatitis). Other clinical information would also be interesting: days from clinical onset to death, other liver enzymes, etc). Although numbers are very small, it would be desirable to include in table 1 a column with the p values comparing patients with fulminant hepatitis B and hepatitis E.*

Patients dying of acute fulminant hepatitis A may be a better control group. However, in India, HAV infection in adults is extremely rare and such patients thus are not available. Hence, we used acute HBV as the control group.

HBV DNA and HEV RNA values are not available. Information on other liver enzymes, serum albumin and duration of illness (from onset to death) has been added to the Table.

Information on P value and statistical comparison of fulminant hepatitis B and E has been added as a foot note to the Table.

- (2) *Minor comments: (1). Page 8, 2.6 Materials and Methods section It was stated "Formalin-fixed, paraffin-embedded liver sections were stained with hematoxylin and eosin, and examined to confirm the presence of acute inflammation in patients with FH, and absence of disease in controls". authors should provide specific pathological character of acute inflammation observed under HE stain. (2). Page 10,*

Results section, first paragraph need revise, should not start with a table title. (3). page 11, results section, 3.3.2. the found of genes differentially expressed in FH-E need detailed description, though these results also can be checked in table 3. (4). Discussion section should focus more on the novel observations and should put the results, also the discussion does not include enough about the meaning of the results.

1. Findings of acute inflammation on HE stain have been added to the text.
2. Page 10, results, first para: Changed so that it does not start with table title.
3. Though the reviewer's suggestion of adding the names of genes and pathways showing differential expression is good, implementing it would lead to a lot of repetition of data in the tables. This will also add to the length of the manuscript. Hence, we would not like to repeat this information here in text. If the reviewer insists, please let us know and we will do this.
4. Paragraphs 4-7, 10 and 11 of the discussion section discuss the implication of our results, and paragraph 12 lists the limitations of our study. The discussion section is already more than 4 pages long and hence it may not be useful to add to it. If the reviewer or the editorial board would like us to discuss any specific aspect, we would surely be willing to do so.

(3) This is an interesting study performed by Naik et al, the authors have investigated the gene expression and immune cells in the liver tissues from Fulminant Hepatitis E and healthy control. The study is carefully designed and executed, appropriate controls are included, the references are up to date and limitations of the study are discussed. Specific comments: 1. A paragraph describing the statistical analysis/software is missing in the methods section. 2. In the results section, author stated that the fold change in selected genes was further validated, however the data is not to be found. Please indicate clearly. 3. Have the authors tried to valid overexpressed gene candidates in HEV samples with IHC? 4. Please explain the significance to include the HBV group?

1. A paragraph with statistical methods has been added.
2. Validation data are already shown as a figure (Fig 3).
3. We did not do validation using IHC.
4. The HBV group was included as a disease control group - i.e. a group which had comparable degree of hepatic injury and inflammation, but did not have HEV infection.

3 References and typesetting were corrected

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

Sincerely yours,



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