September 12, 2019

Prof. Liansheng Ma

Editor-in-Chief

World Journal of Gastroenterology

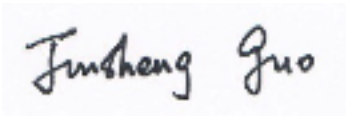
Re: Manuscript NO.: 49835 entitled “A study on serum amyloid A levels in patients with liver diseases”

Dear Prof. Ma,

Thank you very much for your critical review on our manuscript. We herein resubmit a revised version of our article with modifications in response to the reviewer’s comments. The revision was highlighted in red in one copy of the revision. Our point-by-point replies to the comments are enclosed below.

We hope that the amended manuscript is acceptable for publication.

Sincerely yours,



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The following are revisions of our manuscript made in response to the comments of the reviewers:

Reviewer#1

Q1: The authors should include more numerical data into the results section of the abstract. Please provide.

Response:

Thank you very much for your kindly suggestion. Numerical data has been added into the results section of the abstract in the revised manuscript.

Q2: A flow chart of the study should be provided.

Response:

We appreciate the suggestion. A flow chart of the study has been provided into the revised manuscript as Figure 1.

Q3: All of the pharmacological treatments of the patients should be provided and finally included in the multiple regression analysis.

Response:

Thank you for pointing out this important issue. Information on the usage of oral antiviral drugs in CHB patients has been provided and the association of the treatment with SAA level was analyzed.

The disease history of the CHB patients ranged from 1 year to 40 years. No significant difference was found in serum SAA levels between cirrhotic and non-cirrhotic patients (P = 0.537), and between patients with or without oral antiviral drugs (P = 0.634).

Eighteen of the 59 active hepatitis patients and 82 of the 146 inactive hepatitis patients were receiving antiviral therapy. The types and proportions of antiviral drugs used by the patients were Enticavir (ETV) monotherapy 58%, Adefovir (ADV) monotherapy 12%，ETV and ADV combination therapy 2%, Lamivudine monotherapy 6%, Lamivudine and ADV combination therapy 5%, Telbivudine 12%, Telbivudine and ADV combination therapy 2%, TDF monotherapy 3%. Among patients who were receiving antiviral therapy, patients with inactive hepatitis（N=82）had significantly lower blood SAA levels than those patients with active hepatitis (N=18) (Z value = -4.077, P = 0.000) (table 4), albeit their mean level of SAA (6.289±6.042 mg/L) was under the upper normal limit. This may reflect a confounded status of insufficient or ineffective antiviral therapy in these active CHB patients, and in line with the result that patients with active CHB had higher levels of SAA than those with inactive CHB.

For the pharmacotherapy of other liver diseases, patients with autoimmune liver diseases were mainly treated by ursodeoxycholic acid. Five of them were prescribed

with prednisone additionally. Patients with nonalcoholic steatohepatitis and drug-induced liver injury were receiving glycyrrhizic acid, Silymarin or Silibinin, polyene phosphatidylcholine, ursodeoxycholic acid, reduced glutathione treatment. Most patients were using two or more hepatoprotective drugs simultaneously. Patients with liver abscess were treated with third or fourth generation of cephalosporin, third or fourth generation quinolones, metronidazole/ornidazole, or carbapenems. Most patients were receiving two or more antibiotics. Because of the small sample size or the big variation between individuals, we did not further analyze the impact of different therapeutic drugs on SAA levels in various liver diseases.

Reviewer #2:

Q1: The demographics of the patients is not enough (disease duration, antivirals, antiviral treatment duration, drugs, comorbidities etc..). Some studies indicated 'serum amyloid A is expressed primarily in the early phases of disease and might influence progression and/or response to treatment (Serum amyloid A immunohistochemical staining patterns in hepatitis.Piotti KC, Yantiss RK, Chen Z, Jessurun J.Histopathology. 2016 Dec;69(6):937-942. doi: 10.1111/his.13016. Epub 2016 Aug 25). More information and statistics are needed.

Response:

Thank you for your critical review. Detailed information and more statistics have been added in the manuscript on the disease duration, antivirals, antiviral treatment duration, drugs, comorbidities etc.of the CHB patients.

No significant difference was found in serum SAA levels between cirrhotic and non-cirrhotic patients (P = 0.537), and between patients with or without receiving oral antiviral drugs (P = 0.634).

The types and proportions of antiviral drugs used by the patients were Enticavir (ETV) monotherapy 58%, Adefovir (ADV) monotherapy 12%，ETV and ADV combination therapy 2%, Lamivudine monotherapy 6%, Lamivudine and ADV combination therapy 5%, Telbivudine 12%, Telbivudine and ADV combination therapy 2%, TDF monotherapy 3%.

Among patients who were receiving antiviral therapy, patients with inactive hepatitis（N=82）had significantly lower blood SAA levels than those patients with active hepatitis (N=18) (Z value = -4.077, P = 0.000) (table 4).

Patients with inflammatory diseases and systemic diseases have been excluded in this study. As for the complications of liver cirrhosis, we've compared SAA levels between patients with or without ascites, upper gastrointestinal bleeding, hepatic encephalopathy. No statistical difference of SAA levels was found between patients with and without ascites by continuous calibration chi-square test (P=0.080). The OR value of SAA levels above 6.4 mg/L in patients with ascites alone was 3.000 (95% CI: 1.029-8.749, Table 2). This may be due to the slight inflammatory state in the patients with ascites, or the small sample size. Follow-up studies may expand the research by enrolling patients with spontaneous bacterial peritonitis (SBP). There was no significant differences in SAA levels between patients with hepatic encephalopathy

(P=1.000), upper gastrointestinal bleeding(P=1.000), any one of the three complications and those without these comorbidities (P=0.176). Single factor analysis of this study showed that SAA level≥6.4mg/L is not associated with Child-Pugh grades (P=0.068) and Hepatocellular carcinoma (P=1.000) in CHB patients. Please refer to Table 2 for details.

The reference recommended by the reviewer (Serum amyloid A immunohistochemical staining patterns in hepatitis. Piotti KC, Yantiss RK, Chen Z, Jessurun J. *Histopathology*, 2016 Dec;69(6):937-942. doi: 10.1111/his.13016. Epub 2016 Aug 25) do provide valuable information by a clear staining of SAA in hepatitic livers. Functional role of SAA and its regulation in various liver diseases warrant further studies.

Q2: SAA and CRP: 'CRP is not sensitive in the detection of liver injury and dysfunction in clinical practice' Why did you selected CRP?

Response:

CRP is the most commonly used inflammation index in the clinical practice. There are biological and functional similarities of CRP and SAA, that is why we chose CRP as an indicator of inflammation and compared with SAA. Comparative studies have demonstrated that SAA has higher sensitivity and specificity, as well as a broader range of serum level than CRP in some diseases, indicating that SAA may be a more sensitive and better indicator to capture mild inflammation. Moreover, SAA has additional property to stimulate HSCs activities during liver injury and hepatitis, So SAA may be a potential indicator for liver diseases. The reasons above explain why we selected CRP.

Q3: How did you find the cut off 3 mg/l for CRP. Is this reference level of CRP at your laboratory?

Response:

The upper normal limit of CRP as 3 mg/L is provided by the clinical laboratory of Zhong Shan Hospital. We've added this note in the paper.