Response to Reviewers' Comments

July 29, 2013

Dear Editor,

On behalf of my co-authors, I am submitting the revised version of the manuscript authored by Behairy ***et al***. "4044". All the authors have revised the manuscript and approved its contents. Response to reviewers' comments was addressed carefully point-by-point in the following pages and changes in the manuscript are highlighted yellow.

Please find enclosed the edited manuscript in Word format (file name: 4044-review.doc).

Based on the reviewers' comments and the manuscript revision, new statistical analysis yielded a new finding. For that, we believe that the title will be more reflective of the contents if modified as below.

Title: Serum complement C4a and its relation to liver fibrosis in children with chronic hepatitis C

Author: Behairy E. Behairy, Ghada M. El-Mashad, Ragab S. Abd-Elghany, Enas M. Ghoneim, Mostafa M. Sira

Name of Journal: ***World Journal of Hepatology***

ESPS Manuscript NO: 4044

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 The manuscript has been revised for proper English language by a specialized office for scientific writing services.

3 Revision has been made according to the suggestions of the reviewer as indicated below with point-by-point response.

Reviewer: 02444864

Comment 1:

The submitted manuscript (number 4044) entitled “Serum complement C4a does not predict liver fibrosis in children with chronic hepatitis C”, investigates the significance of C4a as a surrogate marker for fibrosis in a children population with HCV infection. The study, although well written, it is not well designed, because the authors have evaluated a surrogate marker which has not yet been established, in a patients setting widely characterized by mild hepatitis and low prevalence of cirrhosis. In addition, the category of severe fibrosis /cirrhosis is totally missing from their cohort, thus rendering their results of limited value. The study has to be enriched with additional cases or it should be temporally restricted to histopathology in association with clinical, biochemical and virological characteristics of HCV infection in Egyptian children.

Answer 1:

The reports for C4 as a marker of liver fibrosis in adults were contradictory and the studies included patients with severe fibrosis up to established cirrhosis. Such high stages of fibrosis and cirrhosis are not common in chronic HCV affecting pediatric age group. Fibrosis stage 4 or higher, according Ishak scoring system, are rarely found except when HCV is accompanied by co-morbidity of the liver (such as autoimmune hepatitis, Wilson's disease, Diabetes etc..) such patients were excluded from our study. The main target of our study was to evaluate C4a as a marker of fibrogenesis which might be of value only in predicting significant fibrosis (Ishak score ≥3) but not the individual fibrosis stages.

Additional Comments

Comment 2

The patients group has to be re-defined because it encompasses not only children but also adolescents.

Answer 2

We used the term children here arbitrarily referring to the pediatric age group which is defined as "those who are up to 18 years old".

Comment 3

Grading is arbitrarily modified and does not correspond to Ishak et al scoring system. The applied scoring resulted in the absence of minimal hepatitis and in an enlarged group of moderate hepatitis giving a wrong impression of hepatitis severity which in this particular setting is minimal and mild in the vast majority of the patients. The categorization has to be as follows: grade 1-3 minimal hepatitis, grade 4-7 mild hepatitis, 8-12 moderate hepatitis and 13-16 severe hepatitis.

Answer 3

The grading was categorized according to Ishak et al, 1995 as instructed by the reviewer. Statistical analysis was performed according the new categorization.

Modifications were made accordingly in the manuscript in methodology, results and discussion sections.

Comment 4

In Table 2 and 3, the total number of patients recorded for fibrosis stage is 29 instead of 30. Most probably F0 is missing. F0 should be validated separately or together with F1.

Answer 4

F0 was added in table 2 for the descriptive purpose, while it was added to mild fibrosis category to be (No / Mild fibrosis) in table 3 and validated together as instructed by the reviewer. Changes were made accordingly in the results and discussion sections.

Minor Comments

Comment 5

Few mistakes in the English langue have to be corrected.

Answer 5

The manuscript was revised for proper English by a specialized office for scientific writing services.

Answer 6

In page 10, 2nd paragraph, the sentence"C4 specific activity appears……" needs a reference.

Answer 6

The reference for this sentence is the same of the following sentence (ref. # 29; ***Dumestre-Perard et al., 2002***). In the revised version, the following sentence "This activation, in an inflammatory context …" was omitted, so the reference will follow the sentence directly.

Reviewer: 00182548

Comments to Authors

The article is interesting. The statistical analysis is good, but the number of patients is small.

Comment 1

There are some small mistakes: spearman's (in my opinion Spearman's is correct) (abstract and statistical analysis) and Complement C4 (not C4a) is a polymorphic serum protein consisting of two isoforms, C4a and C4b (page 4).

Answer 1
Corrected as indicated

Comment 2
In my opinion, the article can be published, but authors should mention in conclusion that the results were interpreted considering the small number of patients.

Answer 2
This information was added to the discussion section as instructed.

Reviewer: 00181536

Comments To Authors

This report exhibited the relationship between serum complement C4a and clinical chracteristics of infantile chronic hepatitis C patients. The objective was interesting, however, the results included only negative data and not highly impacted.

Major

Comment 1
Fibrosis stages are not continuous variables. They must be shown as ordinal variables.

Answer 1
Both fibrosis stage and activity grade were represented as ordinal variables as indicated in tables and in the text.

Comment 2
As adult patients with chronic hepatitis C, the authors are recommended to investigate adult patients to confirm that the experimental procedures are correct.

Answer 2
Four patients with co-morbid liver disease "autoimmune hepatitis with HCV infection" were included in the ELISA assay. Their mean serum level of C4a was 92.71 ± 35.68 mg/L. Two of them had F5 and the other two had established cirrhosis (F6 according to Ishak score). These patients were not included in the study as they did not match the inclusion criteria; nonetheless, they demonstrate the validity of the experimental procedures.

Reviewer: 00004157

Comments To Authors

Based on previous reports supporting a role of C4a as a noninvasive marker of fibrosis in children with hepatitis C, in this paper, Sira et al evaluated whether C4a may represent a marker of fibrosis in Egyptian children with chronic hepatitis C, but they found negative results.

Comment 1

I would suggest to add a power analysis to specify to what extent authors could exclude that C4a is a marker of severe fibrosis, and discuss the results.

Answer 1

The performance of C4a in discriminating patients with significant fibrosis (Ishak score ≥3) was assessed by receiver-operating characteristic (ROC) curve analysis. It had a sensitivity of 76.9% and a specificity of 75%. The new information was added accordingly in the methodology, results (as figure 2) and discussion sections.

4 References and typesetting were corrected

5 Figures are supplied as editable PowerPoint slides

We appreciate the careful review and would like to thank the reviewers for their comments and suggestions that were helpful in revising the manuscript. We believe that the manuscript has significantly improved with the changes made. We hope that our manuscript is now suitable for publication in the ***World Journal of Hepatology.***

Sincerely yours,

Mostafa Sira, MD
Editorial board member ID: 02447059
Department of Pediatric Hepatology
National Liver Institute
Menofiya University, 32511 Shebin El-koom
Menofiya, Egypt.
Tel: (+20)-48-222-2740
Fax: (+20)-48-223-4586
E-mail: msira@liver-eg.org