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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14693review.doc).

Title: Serum proinflammatory cytokines and nutritional status in pediatric chronic liver disease

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

Revision has been made according to the suggestions of the reviewers:

→ **Reviewer 02860585**

(1) *Limits of the study.*

The following paragraph was changed in the text in the Discussion section:

“The limitation of the study may be due to the small number of severe liver disease patients (Child-Pugh C) because all patients were enrolled in the outpatient clinic. Moreover, the study design (cross sectional) did not allow for a medium or long-term follow up to assess the nutritional and clinical outcomes”.

(2) *Definition of cirrhosis should be improved, explaining how laboratory tests can diagnose liver cirrhosis. Furthermore, esophageal varices can be related to non-cirrhotic portal hypertension. Consequently, authors should indicate the number of patients diagnosed by each method.*

We agree that the laboratory tests are not accurate to diagnose liver cirrhosis by themselves. Basically, the diagnosis of cirrhosis was based on histological and/or ultrasonographic, and clinical criteria. We changed the following paragraph in the text in the Methods section:

“In our study, cirrhosis was diagnosed by histological and/or standard ultrasonographic and clinical criteria in patients with chronic liver disease. The histological criteria were the presence of nodular formation and fibrosis on liver biopsy. Ultrasonographic findings were the presence of

esophageal varices on endoscopy and/or ultrasound, showing heterogeneous echogenicity of the liver and signs of portal hypertension. The clinical criteria were hepatosplenomegaly, ascites, hypoalbuminemia and coagulopathy" [1].

Reference:

1 - **Schuppan D**, Afdhal NH. Liver cirrhosis. Lancet 2008; 371(9615): 838–851.

In regard of the esophageal varices *vs* non-cirrhotic portal hypertension, all patients had esophageal varices due to portal hypertension. We also changed the following paragraph in the Results section:

"The study population's median (25th - 75th centile) age was 60 (17-116) months. BA was the main cause of chronic liver disease (72%). Eight patients had no cirrhosis criteria at the time of enrollment. Of all 35 cirrhotic patients, 24 were diagnosed by liver biopsy. From the remaining 11 without liver biopsy, all had ultrasonographic alterations that were compatible with cirrhosis and portal hypertension (splenomegaly and/or esophageal varices) without portal vein thrombosis".

→ **Reviewer 02444959**

(1) *Material and methods: please, briefly describe the biochemical assays performed.*

The following paragraph was changed in the Methods section:

"Blood samples were collected from all patients during the performance of routine tests such as: serum albumin, creatinine, conjugated bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time, international normalized ratio (INR) and CRP. All laboratory tests were executed according to standard operating protocols from the Biochemistry Laboratory of the local institution".

(2) *Results and discussion: Please, describe, show (include in a table) and discuss all the obtained results such as AST, ALT, etc.*

We would like to emphasize that creatinine levels, prothrombin time and INR were needed to calculate the scores used in the study such as Child-Pugh, PELD and MELD. They were not evaluated aiming to assess any association with cytokine levels. Median levels of AST, ALT and CB and all correlations between IL-6 and these laboratory tests were showed in Table 3.

Concerning AST, ALT and CB levels the following sentence was added in the Discussion section:

“Moreover, we also found a positive correlation between IL-6 and routine liver function tests such as AST, ALT and CB”.

→ **Reviewer 00742223**

(1) *Limited number of cases*

We would like to point out that this study sample represents the outpatient population from a tertiary referral center for pediatric liver diseases in the South of Brazil that fulfilled all inclusion and exclusion criteria previously established.

(2) *Nutritional habits (glucose, lipid and protein intake) and social status*

We would like to emphasize that all patients were under a regular nutritional follow up, and dietary intake was evaluated as a systematic approach of the multidisciplinary team at our institution. All patients had a normocaloric median daily adequacy compared to Dietary Reference Intakes (DRI). In regard of the macronutrient distribution from total caloric intake, it was found to be adequate in carbohydrates, proteins and lipids (data not published). In regard of the social status, we did not have a detailed evaluation; however, the sample was obtained from a public hospital with a very homogeneous social status. Nevertheless, we believe that the social status would not influence the proposed outcome.

The following sentence was changed in the Methods section:

“All patients were under regular nutritional follow up and their dietary intake was evaluated as a systematic approach of the multidisciplinary team at our institution”.

(3) *Illness related malnutrition vs non illness related malnutrition vs chronic inflammation in obesity*

We agree that all the studies showed by the reviewer suggest that obesity has currently been characterized as a chronic inflammatory state. Additionally, *non illness related malnutrition* may lead to a low immune response in children and high susceptibility for infections. However, we believe that the rationality of our study is related to the inflammatory activity that has been

suggested as an important component of the pathogenesis of *illness related malnutrition* (secondary to one or more diseases)^[1]. Therefore, we considered that this was our main field of research and thus the theoretical approach adopted for this study.

As we pointed in our Introduction section, several studies have already evaluated the proinflammatory cytokine profiles in children with chronic liver disease, but none of them addressed the possible association between these biomarkers and nutritional status deterioration that we showed in our study. Moreover, to the best of our knowledge, this is the first study in the literature to show the association between IL-6 levels and nutritional risk in children and adolescents with chronic liver disease.

Reference:

1 - **Mehta NM**, Corkins MR, Lyman B, et al. Defining pediatric malnutrition: a paradigm shift toward etiology-related definitions. *JPEN J Parenter Enteral Nutr* 2013;**37**:460-481.

→ **Editor**

The following documents requested were attached to the manuscript:

- (1) Language certificate (edited for grammar)
- (2) Ethics approval document
- (3) Informed consent file
- (4) Conflict of interest statement
- (5) Biostatistics statement
- (6) Copyright transfer

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

Sincerely yours,

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