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Original article

Soluble CD163 in Liver Diseases in Children

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Abstract

Background: Activation of macrophages may be tracked in the blood through the biomarker soluble CD163 (sCD163). The purpose of this research is to determine the significance of soluble CD163 in the severity of liver disease in children. **Methods:** This study was a case control study performed on 128 children divided into cases with chronic hepatitis C (68 cases) and normal control group (60 cases). Patients fulfilling the criteria were evaluated with complete history, clinical examination, and laboratory investigations including ALT, AST and sCD 163 level. **Results:** This study showed that the serum sCD-163 had a significant role in prediction of the liver problem. The mean sCD-163 for patients with chronic hepatitis C was 17.7±7.2 and for controls was 9.8±1. **Conclusions:** The present study shows that the serum sCD-163 had a significant role in prediction of liver damage in patients with chronic hepatitis in children.

1. Introduction:

Activation of macrophages may be tracked in the blood through the biomarker soluble CD163 (sCD163). Kupffer cell activation during inflammation and oxidative stress is reflected by this marker in liver illness, and it is linked to disease severity, prognosis, and treatment responses in a variety of inflammatory liver disorders.(1).

Acute liver failure, acute-on-chronic liver failure, and alcoholic hepatitis are inflammatory and necrotic conditions characterized by very high levels of sCD163, which correlate strongly with illness severity and mortality.(2).

sCD163 is a marker of liver cirrhosis severity. It predicts illness progression and survival with high accuracy, and it correlates with severity levels (MELD, Child-Pugh). Diagnostic accuracy for substantial fibrosis exceeds established diagnostic scores like APRI and FIB-4, and sCD163 rises linearly with inflammation and fibrosis in chronic hepatitis B and C.(3).

Nonalcoholic fatty liver disease (NAFLD) is associated with lower levels of sCD163, yet sCD163 provides valuable diagnostic information for identifying individuals with advanced illness. The growing body of literature on sCD163 in liver disease points to it as a clinically valuable biomarker that contributes to our understanding of illness and its management at every stage.(4).

Currently, ELISA-assays are used to detect SCD163 in scientific studies. Decision limits and prognostic scores need worldwide standardization and, ideally, a switch to automated analytical platforms before they may be used safely in ordinary clinical practice.(5).

Kupffer Cells and Hepatic Inflammation Macrophages serve a crucial role in maintaining liver homeostasis and in the progression of inflammatory liver disease. Liver resident macrophages called Kupffer cells are the body's most numerous kind of tissue macrophage, making up as much as 90 percent of the total. When the liver is damaged, more circulating monocytes are drawn to the site, where they undergo differentiation into tissue macrophages. The buildup of macrophages may be facilitated by the proliferation of resident Kupffer cells, as well. (6). The aim of the study is to evaluate serum levels of soluble CD163 in children with liver diseases and explore its role in disease severity.

2. Patients and Methods:

This study was a case control study carried out at Pediatric department, Beni-Suef university hospital, after approval by the department of Pediatrics, faculty of medicine, Beni-Suef University and after obtaining approval from the local research and ethical committee during the period from December 2020 till the end of June 2021.

One hundred twenty eight Child cases with chronic hepatitis C (68 cases), and normal control group (60 cases).

Antibody identification to HCV antigens using a third-generation enzyme immunoassay technology allowed for the diagnosis of chronic hepatitis C in these patients. The diagnosis was subsequently verified by the use of quantitative PCR to quantify HCV RNA. The presence of fibrosis was then evaluated using a fibroscan.**(7).**

Inclusion criteria:

- Patients of both sexes aged between 3 years to 15 years old.
- Patients proven to have chronic liver disease based on clinical and laboratory characters.

Exclusion criteria:

- Patients with liver disease associated with other chronic diseases, renal, cardiac or rheumatic.

Methods:

The clinical data of the patients fulfilling the inclusion criteria were evaluated as follows:

1) Clinical Data:

- Take a thorough medical history, paying close attention to the patient's age at the beginning of liver disease symptoms, the patient's history of blood transfusions, and the presence of hepatitis signs including jaundice and dark urine.
- Comprehensive physical examination including weight, height, and vital signs taken.

2) Laboratory Investigations:

- Alanine transferase (ALT).
- Aspartate aminotransferase (AST).

Soluble CD163 level by ELISA technique.

Principle of the technique (enzyme linked immunosorbant assay):

This assay uses a particular antibody of the target molecule to detect and quantify a target antigen in a serum sample. An enzyme-labeled antibody is used to detect the antigen-antibody complexes, and the colorimetric

measurement of enzyme activity provides the readout. Substrates that undergo color changes upon enzyme modification are used to quantify enzyme activity. The produced substance is evaluated by how much light it absorbs.

Steps:

Wells coated with pure sCD163 antibody were used to determine pipette standards and serum samples. After covering it with the supplied adhesive strip, it was incubated at 37°C for 30 minutes. The waste liquid was drained, treated with detergent, and finally disposed of. Enzyme Supplement: Add HRP-conjugated reagent by pipette to all wells except the blank. Incubate at 37 degrees for 30 minutes after covering with the supplied adhesive strip. Garbage collection, dishwashing liquid rinse, and final drain. Using for coloring (TMB substrate solution) Add a few drops of chromogen solution A and a few drops of chromogen solution B to each well using a micropipette. Put the accompanying adhesive film over it and leave it at 37 degrees for 15 minutes. Reaction halted by adding stop solution via pipette to each well (as seen by the blue hue becoming yellow). In 15 minutes, you can get an absorbance reading at 450 nm..

Statistical methods:

Data was analyzed using SPSS for Windows (Statistical Package for the Social Sciences) version 25. The following is a description of the variables: Mean and standard deviation were used to describe quantitative variables (SD). Number and percentages were used to describe qualitative factors. Scale variables with non normal distribution were compared across groups using the Mann whitney U test, whereas regularly distributed variables were compared using the independent t test. To determine the best CD163 cutoff for usage in predicting the kind of liver disease relative to healthy controls, a ROC curve analysis was performed.

Correlations between properly distributed variables were determined using Pearson correlation. P-values were used to determine the level of significance of the findings, which were broken down as significant at less than 0.05.

Ethical consideration:

This study protocol was approved by the research ethics committee of Faculty of Medicine of Beni-Suef University number FMBSUREC/04102020/Rasmy. The study protocol was done according to the declaration of Helsinki.

3. Results:

Table (1): Age and sex distribution among the studied groups.

Items	Chronic Hepatitis C (no=68)	Controls (no=60)
Age (years) (mean±SD)	8.3±3.1	5.8±2.9
Sex Males Females	 27(39.7%) 41(60.3%)	 45(75.0%) 15(25.0%)

This table showed that the mean age of children with chronic hepatitis was 8.3±3.1 years and that of healthy children was 5.8±2.9 years.

Table (2): Clinical presentation and examination among the studied groups.

Items	Chronic Hepatitis C (no=68) No(%)
Abdominal pain	3(4.4%)
Ascites	1(1.5%)
Jaundice	12(8.7%)

The abdominal pain was present in 4.4% of cases with chronic hepatitis C, ascites in 1.5%, jaundice in 8.7%.

Table (3): Laboratory parameters among the studied groups.

Items	Chronic Hepatitis C (no=68)	Controls (no=60)	P-value
SGOT	50.1±9.9	16.7±5.3	<0.001*
SGPT	41.2±5.5	15.6±5.1	<0.001*
PT	12±1	11.2±0.5	<0.001*
INR	1.1±0.1	1.23±0.2	<0.001*

**P-value is significant*

This table showed that there was a significant difference between groups regarding SGOT, SGPT, PT, and INR.

Table (4): Comparison between the studied groups regarding the sCD-163 level.

Items	**Chronic Hepatitis C (no=68)**	**Controls (no=60)**	**P-value**
SCD 163 (ng/ml)	17.7±7.2	9.8±1	<0.001*

**P-value is significant*

This table showed that there was a significant higher level of SCD163 in chronic vs controls.

Table (5): Comparison between patients with chronic hepatitis C with fibrosis, patients with chronic hepatitis C without fibrosis and control.

Items	**Chronic Hepatitis C with fibrosis (no=33)**	**Chronic Hepatitis C without fibrosis (no=35)**	**Controls (no=60)**	**P-value**
Age (years) (mean±SD)	8.7±2.7	8±3.3	5.8±2.9	**----**
Sex Males Females	 21(63.6%) 12(36.4%)	 6(17.1%) 29(82.9%)	 45(75.0%) 15(25.0%)	**---**
SCD 163	**21.3±8.1a**	**12.9±2.2b**	**9.8±1c**	**<0.001***
SGOT	62.7±6.5a	24.5±9b	16.7±5.3b	<0.001*
SGPT	54.1±5.1a	19.8±6.6b	15.6±5.1b	<0.001*

**P-value is significant*

Post Hoc pair-wise comparison was illustrated using small letters (different letters denote significant difference between groups)

This table showed that there was a significant higher level of SGOT&SGPT& SCD 163 in chronic hepatitis with fibrosis than chronic hepatitis without fibrosis and control groups. SCD 163 was also higher in patients without fibrosis than controls but SGOT & SGPT did not differ between patients without fibrosis and controls.

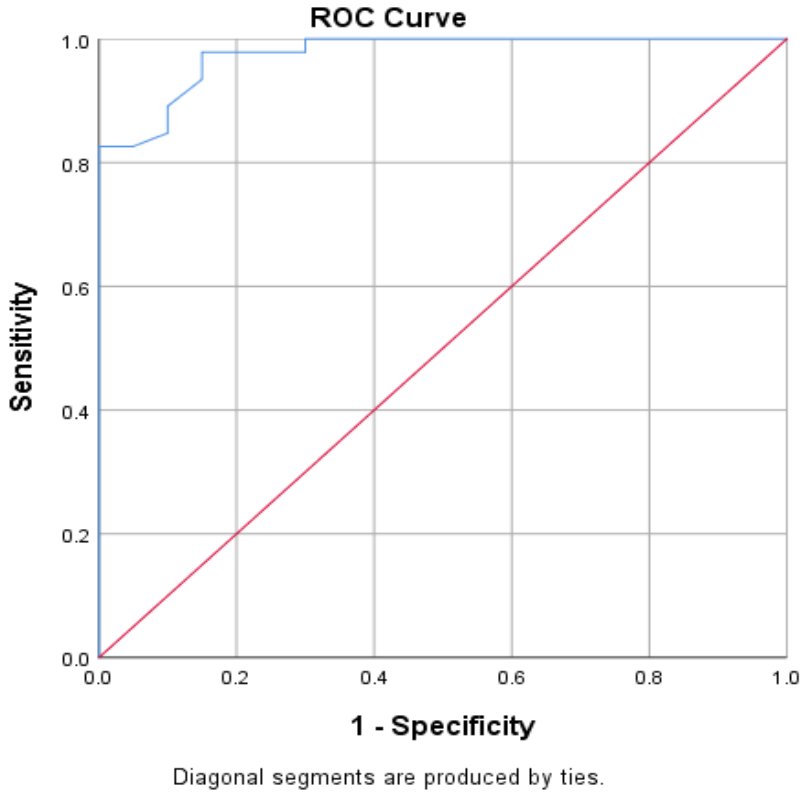

Figure (1): Receiver Operating Characteristic curve for prediction of chronic hepatitis using serum sCD-163 (no=68) versus healthy controls (no=60).

Table (6): Sensitivity, specificity, PPV and NPV of serum sCD-163 in detection of liver disease.

Items	**Chronic hepatitis C vs Control**
P-value	<0.001*
Cut off	≥11.2
AUC	0.976
Sensitivity	89.1%
Specificity	90%
PPV	90%
NPV	90.5%

This table and figure showed that the serum sCD-163 had a significant role in prediction of the liver problem. At a cut off more than or equal 11.2, sCD-163 can predict the chronic hepatitis (vs control) with 89.1% sensitivity and 90% specificity.

4. Discussion:

The activation of macrophages is crucial to the progression of liver inflammation and fibrosis in liver disorders. Over 80% of the body's macrophage population resides in the liver, and they are called Kupffer cells. Injured livers attract innate effector cells upon Kupffer cell activation.**(8).**

Inflammatory stimuli induce the release of CD-163 into the bloodstream. CD-163 is expressed ubiquitously and functions as a scavenger receptor for hemoglobin and haptoglobin. Healthy people also have soluble CD-163 in their blood plasma. CD-163 levels are greatest in individuals who ultimately die from their liver illness, and they rise in tandem with the severity of the disease.**(9).**

This study was conducted at Beni-Suef University hospital on children with liver diseases to evaluate serum levels of soluble CD-163 in children with liver diseases and explore its role in disease severity.

Regarding the demographic data of the cases under the study, children with chronic hepatitis had an average age of 8.3±3.1 years with females representing 60.3% of cases and that of healthy control children was 5.8±2.9 years and most of them were males (75%).

This study showed that abdominal pain was present in 4.4% of cases with chronic hepatistis C. By examination, hepatosplenomegaly was detected in76.5% of cases with chronic hepatitis C had hepatomegaly and 47.1% had splenomegaly. Ascites was present in cases chronic hepatitis C only in 3 1.5%.

Similar results were reported by **Usman *et al.*, (2022)** in their study about outcome of treatment of children with chronic viral hepatitis. They reported that of 30, 16 patients (26.6%) had mild symptoms of anorexia, nausea, abdominal pain, constipation, fatigue, and body aches. The rest of the patients (73.4%) had no symptoms **(10).**

Hepatosplenomegaly was also found to be more common in those with chronic hepatitis, as shown by the results of the current investigation. According to Roy et al. (2013), who examined 51 Indian infants with chronic liver illness, hepatomegaly was evident in 92.16 percent of patients, whereas jaundice (47.06%), poor growth (35.29%), and abdominal distension (23.53%) were the most common presentations.**(11).**

Hepatosplenomegaly was found in 26.2% of patients in a study of the etiology and clinical presentation of chronic liver illnesses in children by Lodhi et al. (2020). Similar but

somewhat different findings were reported in this investigation compared to those reported in the literature. Possible causes for this variation include selection criteria.**(12).**

Patients with hepatic dysfunction might benefit greatly from laboratory tests that evaluate their liver function. The liver is responsible for breaking down nutrients including glucose, protein, and fat. Biochemical markers of liver dysfunction may include metabolic pathway end products and certain enzymes that are very sensitive to the presence of a malfunction.**(13).**

The laboratory results for cases under the study showed that the mean SGOT level for cases with chronic hepatitis C was 50.1±9.9 and for SGPT was 41.2±5.5.

For patients with chronic hepatitis C associated with fibrosis, the mean SGOT level was 62.7±6.5 and the mean SGPT level was 54.1±5.1. Unlike patients with chronic hepatitis not associated with fibrosis in which the mean SGOT level was 24.5±9 and the mean SGPT level was 19.8±6.6.

These results are in line with those found by Hyder et al. (2013), who examined the correlation between ALT, AST, ALP, and GGT in liver disorders and found that ALT levels are higher than AST levels in viral hepatitis.**(14).**

Children with chronic hepatitis showed slight elevation in liver enzymes and this elevation was more in children with chronic hepatitis associated with liver fibrosis indicating more hepatic damage. Matching with the results was that reported by **Cybulska *et al.*, (2011)** who reported that children with chronic hepatitis had mild elevation of liver function tests with a median AST of 62 IU/mL (interquartile range: 37– 113 IU/mL) and a median ALT of 66 IU/mL (interquartile range: 32–105 IU/mL) **(15).**

Schwarz *et al.*, (2015) also demonstrated in their study that children with chronic hepatitis B had slight elevation in ALT and AST levels. The mean AST was 43.8 U/L and the mean ALT was 53.3 U/L **(16).**

This study showed that the serum sCD-163 had a significant role in prediction of the liver problem. The mean sCD-163 was17.7±7.2 for patients with chronic hepatitis C with a significant diference compared to the healthy controls. At a cut off more than or equal 11.2, sCD-163 can predict the chronic hepatitis C (vs control) with 89.1% sensitivity and 90% specificity.

Soluble CD-163 as a measure of fibrosis regression in chronic HCV patients: these findings are consistent with those published by Isaac et al.,

(2019) **(17).** They found that those with chronic HCV had a higher sCD-163 level than healthy controls. Furthermore, this is consistent with the findings of Mascia et al. (2017), who found that sCD-163 plasma concentrations were higher in HCV patients than in controls, indicating systemic immune activation and inflammation and demonstrating that sCD-163 plays a crucial role in the pathogenesis of HCV infection.**(18).**

Soluble CD-163 levels were also higher in patients with advanced fibrosis than in early fibrosis patients, these results are in accordance with **Kazankov *et al.*, (2014) (19)**, and with **Andersen *et al.*, (2014)** who found that sCD-163 levels were elevated in patients with HCV-related cirrhosis compared to those with minimal or no fibrosis showing that sCD-163 may play a role in assessing the severity of hepatitis **(20).**

Increased levels of sCD-163 have been linked to worse disease outcomes and prognoses in studies of both acute liver failure and chronic inflammatory liver disorders. The sCD-163 score also outperforms the more common FIB4 and APRI scores in predicting liver fibrosis. Patients with alcoholic hepatitis have been shown to have much higher than normal levels of sCD-163, and a high sCD-163 concentration has been linked to an increased risk of death. Patients with acute liver failure, and in particular those with a fatal result, have been reported to have the highest sCD-163 levels.**(21).**

5. Conclusions:

The present study shows that the serum sCD-163 had a significant role in prediction of liver damage. As in children with chronic hepatitis at a cut off ≥11.2ng/ml it can

Recommendations:

This study reommends that further studies should be performed on larger sample size to ensure the accuracy of the results. In addition, further studies should be performed on different causes affecting the liver and compare between them. Also, different biomarkers and cytokines should be assessed and comparing between them alone or combined in further studies to reach the most accurate one not only as diagnostic biomarker but also, as a prognostic one.

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