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Cytokines in Chronic Hepatitis C Liver Diseases: Interleukin 20

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ABSTRACT

Background: The Interleukin (IL-20) cytokine subfamily is a major IL-10 cytokine subfamily that is thought to produce a role in the response to hepatic injury. The IL-22 cytokine is the most studied of this family to the inflammatory imbalance of the liver, and it primarily serves bi-role, it is supposed to increase the susceptibility to HCC development.
Objective: We aimed to study the relationship between IL-20 serum levels and the clinical parameters of chronic HCV-associated diseases.
Patients and Methods: In this case-control study, a total of 3 groups included Chronic hepatitis C, liver cirrhosis, and HCC post HCV patients (15 in every group) compared by a healthy group concerning serum IL-20 assay. Clinical features, laboratory, radiological elements, and CLD scoring were evaluated and compared with IL-20.
Results: The April and FIB-4 scoring show a significant difference (p-value < 0.001) between the chronic hepatitis c patients and both the cirrhosis and HCC group, with no significant difference between cirrhosis and HCC groups. There was a significant difference between the three groups regarding the child. Pugh classification (HCC group by 40% as both child B and C, while 20% child A). In both HCC and cirrhosis groups, the IL-20 level is not related significantly to the first presenting symptom.
Conclusions: Newly studied cytokines such as IL-20 are easy, cheap ELISA tests that can be useful in assessing the related clinical parameters of chronic liver diseases.
Keywords: Cytokines, Interleukin, IL-20, Hepatitis C, Liver cirrhosis, Hepatocellular carcinoma.

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for the common cause of primary liver cancers and is the principal cause of cancer-linked death worldwide. Infections with the HBV or HCV and alcohol use are the most important risk factors [1].

Unsettled chronic inflammation is a leading feature of HCC, regardless of the etiology. Local activation of cell populations in the liver in response to organisms and/or tissue injury may initiate a linked and coordinated cascade process, followed by immune cell infiltration, and eventually repair of the organ. The release of soluble factors, such as cytokines, occurs as a result of this fine coordination of events [2].

Cytokines have been studied as potential biomarkers for predicting different stages of HCC and for additional understanding of HCC formation mechanisms. The initial inflammatory response in the liver is unresolved in the presence of HCC-promoting risk factors, and so, the unbalanced expression of cytokines promotes a persistent healing response [3].

By enhancing hepatocyte proliferation and regeneration, which can lead to mutagenesis and set the stage for HCC development, this response may lead to the sequential development of inflammation and eventually HCC. Once HCC has formed, cytokines released by the tumor or immune cells can act on the malignant lesion to promote tumor survival through a variety of mechanisms [4].

Other interleukins in the IL-20 subfamily have the common function of communicating with leukocytes and epithelial cells in different tissues such as the liver. They are crucial in controlling tissue regeneration after injury, promoting survival, and inhibiting apoptosis of epithelial cells [5].

The work aims to study the relationship between IL-20 serum levels and the clinical parameters of chronic HCV-associated diseases.

SUBJECTS AND METHODS

The study design

This case-control study was carried out at Zagazig University Hospitals at the Department of Tropical Medicine from March 2020 to July 2021. Three diseased groups related to HCV infection 15 patients in each group (HCC, Cirrhosis, and Chronic hepatitis) compared to 15 healthy participants in a controlled group.

The study included patients of HCV infection with age more than 18 years old (positive antibody by ELISA and quantitative PCR) and excluded other causes of chronic liver diseases as autoimmune, other chronic liver viral diseases HBV (excluded by HBV surface Antigen), known metabolic, autoimmune diseases (excluded by autoantibodies were negative), and known vascular diseases (Ultrasound Doppler).

Liver cirrhosis was confirmed by history, clinical, laboratory, and radiology evaluation. HCC was evaluated by alpha-fetoprotein and tri-phasic CT, and extra-hepatic malignancy or metastases were excluded. Pregnant and lactating females were also excluded.

Methods:

Complete history, detailed clinical examination, routine investigations, radiological assessment (US (Sonoscape c11) and tri-phasic CT scanning (Philips Ingenuity 128)) and the studied interleukin-20 assay (3-

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5mL of peripheral blood sample was withdrawn and measured by ELISA Glory Science, USA kit) were determined. Liver diseases were scored by **calculat**ing the **FIB4, APRIL Child-Pugh classification.**

Ethical consent:

Approval of the study was obtained from Zagazig University Academic and Ethical Committee. After explaining our research objectives, written informed consent was obtained from all study participants. This study was conducted in compliance with the code of ethics of the world medical association (Declaration of Helsinki) for human subjects.

Statistical analysis

The collected data were coded, processed, and analyzed using the SPSS (Statistical Package for Social Sciences) version 20 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test ($\chi 2$) to calculate the difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare two independent groups of normally distributed variables (parametric data). P-value < 0.05 was considered significant.

RESULTS

The demographic features

The studied patients were classified into 4 groups (group 1(HCC), 2(cirrhosis), 3(chronic HCV), and 4(healthy control), with no significant difference between the studied groups regarding age and sex.

The inflammatory scoring

The April and FIB-4 scoring show a significant difference (p-value < 0.001) between the chronic hepatitis C patients (3) 0.38 ± 0.14, 1.07 ± 0.31, and both the cirrhosis (group 2) 1.78 ± 0.69, 5.31 ± 1.77 and HCC (group1) 1.95 ± 0.94, 5.11 ± 2.2, with no significant difference between cirrhosis and HCC groups (**Table 1).**

Table (1): The difference in the diagnostic scores among all studied groups

	Group 1 N=15	Group 2 N=15	Group 3 N=15	KW test	P-value
APRI score	1.95 ± 0.40*	1.78 ± 0.35**	0.38 ± 0.081	21.3	<0.001
FIB-4	5.11 ± 1.2*	5.31 ± 1.3**	1.07 ± 0.24	22.1	<0.001

HS: High significant p-value <0.001 KW: Kruskal-Walis test of significance

*There was a significant difference between groups 1 &3.

**There was a significant difference between groups 2 & 3, while there was no significant difference between groups 1 & 2.

Child-Pugh Classification

There was a significant difference between the three groups regarding the child. Pugh classification presented in the HCC group by 40% as both child B and C, while 20% as child A. the liver cirrhosis group scored 60% as child B, while 20 % as both A and C classification (**Table 2).**

Table (2): The difference in Child. Pugh classification among all studied groups

	Group I N=15	Group II N=15	Group III N=15	KW test	P-value
CHILD score	**N (%)**	**N (%)**	**N (%)**		
A	**3 (20%)**	**3 (20%)**	**15 (100%)**	**28.1**	**<0.001**
B	**6 (40%)**	**9 (60%)**	**0**		
C	**6 (40%)**	**3 (20%)**	**0**		

The tumor characteristics

The tumor characteristics in the studied HCC group were 86,7% single lesion, 53,3% more than 2X2 cm, no PVT, and 86.7% in the right lobe (**Table 3).**

Table (3): The Tumor characteristics among the patients of group 1 (HCC)

Characters		
	N	%
Tumor number		
1	**12**	**86.7**
2	**2**	**13.3**
Tumor size		
2 * 2 cm	**7**	**46.7**
>2 *2 cm	**8**	**53.3**
PVT		
No	**15**	**100**
Lobe		
Right	**12**	**86.7**
Left	**3**	**13.3**

The first presenting symptom and IL-20

In both HCC and cirrhosis groups, the IL-20 level is not related significantly to the first presenting symptom with the highest level in the HCC group in the patients presenting with abdominal pain Il-20 mean/SD 25.4 ± 1.9 pg/ml while, the highest IL-20 level was 38.9 ± 0.11 ng/ml as abdominal enlargement in liver cirrhosis group (**Table 4).**

Table (4): The relation between the first presenting symptom and IL-20 level among the studied cases

	Group 1 N=15	**Group 2 N=15**
	Mean ± SD	**Mean ± SD**
Asymptomatic	**N=0**	**N=3 20.4 ± 5.1**
Abdominal pain	**N=3 25.4 ± 1.9**	**N=0 27.5 ± 6.32**
Hematemesis	**N=6 18.6±4.32**	**N=6 28.99 ± 6.97**
Abdominal enlargement	**N=2 22.9 ± 4.14**	**N=2 38.9 ± 0.11**
Hepatic encephalopathy	**N=4 24.4 ± 3.62**	**N=4 26.3 ± 7.32**
Significant test	**KW-test= 0.93**	**F- test= 3.46**
P value	**0.82 NS**	**0.06 NS**

The clinical parameters and IL-20

IL- 20 level related to the clinical examination is highly significant in ascites in both HCC (no, mild, moderate amount related median/ range15.8 (2.8-13.5), 23.3 (19.9-25.8), 24.8 (23.8-29.3)pg/ml, p-value 0.007) and Cirrhosis groups (no, mild, moderate ascites amount related mean/ SD 21.5 ± 2.99, 33.7 ± 4.63, 38.9 ± 0.11pg/ml, p-value<0.001). Splenomegaly-related IL-20 level is significant in HCC but, not in the Cirrhosis group. In contrast in the cirrhosis group, IL-20 level is significant-related to Jaundice and LL edema in addition to ascites p-value (0.007, 0.001, and <0.001 respectively) **(Table 5).**

Table (5): The relation between the clinical finding and IL-20 level among the studied cases

	Group I N=15	P	Group II N=15	P
	IL-20 Median (Range)		IL-20 Mean ± SD	
Hepatomegaly	**23.9 (2.8-29.3)**	1.00#	**27.4 ± 6.3**	0.85*
No	**24.2 (14.97-26.7)**	NS	**28.2 ± 6.88**	NS
Splenomegaly	**25.8 (23.2-29.3)**	**0.02#**	**30.9 ± 7.1**	0.07*
No	**17.5 (2.8-27.11)**	**S**	**23.4 ± 5.6**	NS
Jaundice	**24.2 (2.8-29.3)**	0.96#	**36.1 ± 3.31**	**0.007***
No	**23.9 (14.97-27.99)**	NS	**25.8 ± 4.61**	**S**
LL edema	**23.9 (2.8-29.3)**	0.51#	**33.5 ± 8.31**	**0.001***
No	**25.4 (13.5-27.99)**	NS	**21.5 ± 5.23**	**S**
Ascites				
No	**15.8 (2.8-13.5)**	**0.007****	**21.5 ± 2.99**	**<0.001$**
Mild	**23.3 (19.9-25.8)**	**S**	**33.7 ± 4.63**	**HS**
Moderate	**24.8 (23.8-29.3)**		**38.9 ± 0.11**	

Median (Range): nonparametric measures
HS: High significant p-value <0.001 NS: not significant p-value >0.05 S: Significant P-value<0.05
*: t-test of significance **: Krusskal-walis test $: F-test (ANOVA) #: Mann-Whitney test.

DISCUSSION

It is currently unclear detailed how HCV causes liver inflammation, develops cirrhosis, and progresses to hepatocellular carcinoma. Although the function of cytokines in the onset, progression, and/or prevention of lesions has been demonstrated in the development of liver disorders by HCV, these processes remain poorly assumed. There are few studies that evaluate the cytokines in the chronic liver diseases and nearly no studies that studied the IL-20 in liver diseases-related to HCV infection. This study compares the relation between IL-20 serum level and the clinical parameters of chronic HCV associated diseases.

This case-control study included 60 participants and was divided according to HCV infection state. The control group was 15 healthy volunteer subjects, while the diseased (case- group) re-divided according to the liver texture, functions, and presence of focal malignant lesions into three groups (group 1(hepatocellular carcinoma), group 2(liver cirrhosis), and group3 (chronic hepatitis C)).

The comprehensive history, the presented symptoms, and the examined signs were detailed for all the patients, routine laboratory tests, tests to exclude other viral liver diseases than HCV and autoimmune diseases (HBV antigen, ANA, ASMA), alpha-feto protein and radiological assessment (ultrasound and tri-phasic CT) secondary liver metastasis and distant spread, also excluded. The studied patients showed no difference between them as regards age, sex, body mass index, and other chronic diseases.

The inflammatory scoring

The April and FIB-4 scoring – related IL-20 level can be useful in the distinction between the chronic hepatitis C patients and both the cirrhosis and HCC, but not differentiate between cirrhosis and HCC groups that, in agreement with **Lie *et al.*** [6] that concluded that, in chronic HCV patients in China, FIB-4 is an appropriate diagnostic indicator of liver cirrhosis and HCC (**Table 1**).

Child-Pugh Classification

There was a difference between the HCC, liver cirrhosis, and chronic hepatitis C regarding the child. Pugh classification presented in the HCC group by 40% as both child B and C, while 20% as child A. the liver cirrhosis group scored 60% as child B, while 20 % as both A and C classification. This is close to **Sharaf *et al.*** [7] who found that most HCC are decompensated in Child C (**Table 2).**

The tumor characteristics

The tumor characteristics of HCC in this study were mostly single lesions (86.7%), more than 2cm, no PVT, and mainly in the right hepatic lobe (**Table 3).** This was in agreement with **Elkanwey *et al.*** [8] who found that most of the studied HCC characterizes in Egyptian people mainly in the right lobe more than 80% and single lesion in more than 50%.

The first presenting symptom relation to IL-20 level

HCC group was presented with abdominal pain associated with IL-20 median level of 25.4 pg/ml (more than the cutoff 15 pg/ml) while, the highest IL-20 mean level related to abdominal enlargement in the liver cirrhosis group was 38.9 pg/ml. **Table 4**

The clinical parameters and IL-20

IL- 20 level-related clinical examination is useful in detecting the association of ascites in both HCC (no, mild, moderate amount related median15.8, 23.3, 24.8 pg/ml) and Cirrhosis groups (no, mild, moderate ascites amount related mean 21.5, 33.7, 38.9 pg/ml, p value<0.001). Splenomegaly-related IL-20 level is significant in HCC but, not in the Cirrhosis group. In contrast in the cirrhosis group, IL-20 level is significant-related to Jaundice and LL edema in addition to ascites. Studies evaluated IL-20, IL-22, and anti-IL-20 antibodies in acute liver injury, BMI, and HCC growth respectively recorded the association between these cytokines and liver fibrosis, increased BMI, and the usefulness of anti- IL20 in inhibition of HCC growth [9-11].

CONCLUSION

New cytokines such as IL-20 are an easy, cheap ELISA test that can be useful in assessing the related clinical parameters of chronic liver diseases.

Conflict of interests: None
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