IL-1α and TGF-β1 as non-Invasive Liver Fibrosis Markers of Chronic Liver Injury among Chronic Liver Diseases Patients in Sharkia Governorate, Egypt

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Background and study aim: Proper assessment of liver fibrosis and cirrhosis by non- invasive procedures are needed before appropriate management. This study aimed at detecting the role of IL-1α and TGF-β1as non-invasive liver fibrosis markers in chronic liver diseases.

Patients and methods: The subjects included in this study were divided into 4 groups. Group1: 15 chronic HCV patients, group 2: 15 HCC patients, group 3: 15 patients with NAFLD, group 4: 15 healthy control subjects. Serum IL-1α and TGF-β1 measured by ELISA to patients and control groups with calculation of FIB-4 and APRI score.

Results: IL-1α and TGF-β1 were significantly high in all hepatic patients compared to control group. The highest level of serum TGF-β1 was in HCC patients. There was a positive correlation between serum IL-1α and ALT, AST, ALP and HCV RNA by PCR. There was a negative correlation between IL-1α and TGF-β1. There was a highly significant positive correlation between TGF-β1 and FIB4, APRI score, ALP and AFP.

Conclusion: Chronic liver diseases including HCV, HCC and NAFLD were associated with higher levels of IL-1α and TGF-β1 than healthy subjects.

INTRODUCTION

Liver fibrosis involves the deposition of excess extracellular matrix (ECM) proteins in the liver parenchyma as a response to different hepatic insults. Many kinds of cells participate in hepatic fibrosis. The most important one is the hepatic stellate cells which are activated to Myofibroblasts **[1]**. Also, hepatic macrophages or Kupfer cells are activated and produce various chemokine which attract leucocytes that release pro-inflammatory cytokines; Interleukins and TNF-α **[2]**. Interlukin-1alpha (IL-1α) is an inflammatory cytokine that has a significant role in acute and chronic inflammation and may be used as a marker of hepatic injury **[3].**

Evaluating liver fibrosis has an important role in individualizing management of chronic liver diseases. Liver biopsy was used as the standard of care in assessment of liver fibrosis and necrosis [5]. Non-invasive methods had been tried for the last 2-3 decades to replace liver biopsy in evaluating hepatic inflammation, necrosis and fibrosis **[6]**. Non-invasive methods include radiography-based markers like transient elastography (TE) and/or serum-based markers**.** Serum markers are either direct markers of ECM metabolism (e.g. collagens, Metalloproteinases) or indirect markers of liver dysfunction (e.g. albumin, platelets, prothrombin time) **[7].**

This study aimed at assessing the role of IL-1α and TGF-β1 in subgroups of chronic liver diseases (chronic HCV, NAFLD and HCC) in Sharkia governorate, Egypt.

SUBJECTS AND METHODS

Study design: case control.

Patients: This case control study was carried out in the Tropical Medicine and internal medicine Departments, Zagazig university hospitals, Egypt during the period from October 2017 to April 2018. A total of 60 subjects were included in this study.

They were classified into the following groups:

Group I: included 15 chronic HCV patients diagnosed by HCV Abs positivity and positive HCV RNA. **Group II:** included 15 HCC patients diagnosed by characteristic criteria in Triphasic CT. **Group III:** included 15 nonalcoholic fatty liver disease patients diagnosed by history taking, abdominal ultrasound, lab. After exclusion of other cause of liver affection

Group IV: included 15 apparently healthy subjects as a control group.

Inclusion criteria

All Patients attending the outpatient clinic of the Tropical medicine and internal medicine departments fulfilling these criteria: any sex, age >18 years, known chronic HCV, HCC, NAFLD, were offered to participate in the study.

Exclusion criteria

Patients with any possible affection of hepatic inflammation & fibrosis were excluded and this include:

- Patients with sepsis or spontaneous bacterial peritonitis.
- Patients with Acute hepatitis.
- Hereditary liver diseases.

All subjects were subjected to:

A- Full history taking and clinical examination.

B- Pelvi-abdominal ultrasound.

C- Laboratory investigations including:

1- Complete blood picture.

2- Serum creatinine and BUN.

3- Liver biochemistry (Serum albumin, S bilirubin, AST, ALT, ALP).

4- PT, PTT, INR.

5- HCV Antibodies and HBs Ag.

Special investigation including:

- Measurement of serum IL-1α and TGF-β1 by ELISA.
- HCV PCR for HCV antibodies-positive patients.
- Serum alpha fetoprotein (AFP).
- Triphasic Computerized Tomography of the abdomen.
- Measurement of serum ANA, SMA, AMA whenever needed.
- Calculating FIB-4 and APRI scores.

$$\text{FIB-4}=\frac{\text{Age(Years)xAST(U/L)}}{\text{PlateletCount}(10^9/\text{L})\text{x}[\text{ALT(U/L)}]^{1/2}}$$

$$\text{APRI}=\frac{\text{AST}(/\text{ULN}^*)}{\text{PlateletCount}(10^9/\text{L})}\text{x}100$$

Statistical analysis:

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 24. Chi-square was used to examine the relation between qualitative variables. Quantitative data were expressed as mean±SD. One –way ANOVA and Kruskal-Wallis test were used to compare quantitative data. Post hoc test for multiple comparisons using LSD and Dunn test for Parametric and non-parametric variables respectively and Spearman correlation tests were used for correlation of non-parametric variables. Significance was defined as $P<0.05$.

RESULTS

In this study, 60 subjects were included with mean age 50.6 ± 8.5 years, 60% of them were female and splenomegaly was significantly higher in HCC and HCV patients than in other groups (Table 1)**.** It is obvious that patients with HCV infection and hepatocellular carcinoma had significant liver affection reflecting on both ultrasonic and laboratory parameters, as splenomegaly, decrease in hemoglobin and platelets count, increase of serum bilirubin, INR, higher in FIB4 and APRI scores than NAFLD and control groups (Table 2).

It is obvious also from the current study that the studied markers (IL-1α and TGF-β1) are

associated with liver affection, they are higher in all form of liver affection studied when compared to control groups (Table 3). IL-1α is a marker of inflammation, seems to have a correlation with markers of liver inflammation like ALT, AST and alkaline phosphatase. However, TGF-β1, as a marker of fibrosis, was associated with markers of significant hepatic fibrosis represented by significant positive correlation with FIB4 and APRI scores **(Table 5).**

Table (1): Demographic and clinical features of the studied patients.

		HCV N: 15	HCC N: 15	NAFLD N: 15	Control N: 15	P value
Gender	**Male**	6 (40%)	5 (33.3%)	6 (40%)	7 (46.7%)	0.612
	Female	9 (60%)	10 (66.7%)	9 (60%)	8 (53.3%)	
Spleen	**Normal**	11 (73.3%)	9 (60%)	13 (86.7%)	15 (100%)	0.04
	Enlarged	4 (26.7%)	6 (40%)	2 (13.3%)	0 (0.0)	
Liver	**Normal**	10 (70%)	0 (0.0)	0 (0.0)	15 (100%)	0.001
	Fatty	0(0.0)	0 (0.0)	15 (100%)	0 (0.0)	
	Cirrhotic	5(30%)	15 ()	0 (00)	0 (0.0)	

Table (2): Comparison of laboratory values among studied groups.

	HCV	HCC	NAFLD	Control	P
CBC					
WBCs *	4250	6400	6500	6000	0.415
Hb (g/dl)	10.8±0.9	12.5±1.9	12.2±0.8	13.5±0.5	0.001
Platelets	170±70	120±49	284±57	239±29	<0.001
LFTs					
S.albumin	3.4±0.5	3.0±0.9	3.8±0.3	4±0.2	0.004
ALT	43.8±18.8	39.6±22.8	33±15.2	15.3±5	0.014
AST	67±65	50.5±25.5	31±8.8	15±2	0.035
ALP	173.7±51.7	171±90	130.7±43.6	78.3±12.9	0.008
T.bilirubin (mg/dl)	1±0.4	2.2±2	0.9±0.1	0.8±0.1	0.03
INR	1.2±0.2	1.4±0.2	1.1±0.1	1±0	0.006
AFP	8(2.9-12)	4237(2.5-23744	2(1-4)	2(1-3)	0.002
KFTs					
s.Creatinine (mg/dl)	1.1±0.3	1.3±0.5	1±0.2	0.9±0.1	0.133
FIB4 and APRI score					
FIB4 *	2.3(0.8-5.4)	4.8(2.2-17.1)	1(0.7-1.7)	0.7(0.5-0.9)	<0.001
APRI score *	1(0.3-2.3)	1.8(0.5-8.9)	0.4(0.2-0.6)	0.2(0.1-0.3)	0.02

Table (3): Comparison of serum IL-1α and TGF-β1 values among studied groups.

	HCV	HCC	NAFLD	Control	Test	P
IL-1α (ng/l)	5.7(2.8-16.6)	3.4(0.7-12)	4.5(0.7-14)	1(0.5-3)	1.9	0.004
TGF-β1 (ng/ml	13(7-20)	16.3(5.5-50)	12(1-15.5)	3(0.9-4.5)	2.2	0.005
	HCV vs HCC	**HCV vs NAFLD**	**HCV vs control**	**HCC vs NAFLD**	**HCC vs control**	**NAFLD vs control**
IL-1α (P value)	0.292	0.971	0.001	0.76	0.017	0.003
TGF-β1 (P value)	0.05	0.505	0.03	0.027	<0.001	0.076

Table (4): Correlation between serum IL-1α level and certain studied parameters.

IL-1α (ng/l)	r	P
S.albumin	-0.048	o.777
ALP	0.515	0.001
ALT	0.587	<0.001
AST	o.646	<0.001
HCV RNA	0.95	<0.001
T.bili (mg/dl)	0.192	0.255
AFP	-0.991	<0.001
INR	0.177	0.001
TGF-β1 (ng/ml	-0.996	<0.001
FIB4	-0.238	0.156
APRI score	-0.202	0.23

Table (5): Correlation between serum TGF-β1 level and certain studied parameters.

serum TGF-β1	**r**	**P**
S.albumin	-0.077	0.651
ALP	0.51	0.001
ALT	-0.296	<0.001
AST	-0.362	<0.001
T.bilirubin (mg/dl)	0.21	0.213
INR	0.154	0.363
AFP	0.988	<0.001
HCV RNA	-0.949	<0.001
FIB4	0.860	<0.001
APRI score	0.870	<0.001

DISCUSSION

Assessment of hepatic fibrosis is critical for treatment decision and that is why different methods are used for its assessment. Liver biopsy, the gold standard in evaluating hepatic fibrosis, is associated with many complications and hence non-invasive markers are needed. In this study we selected 2 non- invasive markers to be studied. IL-1α as a marker of inflammation and TGF-β1 as a marker of fibrosis were used to cover the spectrum of liver affection from inflammation to fibrosis.

In the current study IL-1α was significant higher in all chronic liver disease patients compared to their matched controls. The highest level of IL-1α was observed in HCV and Non-Alcoholic Fatty Liver Disease (NAFLD) patients followed by HCC patients. Furthermore, IL-1α had a significant positive correlation with HCV-RNA in both HCV and HCC groups and with ALT and AST levels in all included patients.

Two questions ….. First, why there was no significant difference in serum levels of IL-1α in HCV, HCC and NAFLD groups. This can be explained by presence of elevated liver enzymes indicating ongoing hepatic injury in overall hepatic patients, beside that HCC group involved some patients with active HCV infection. Second, why IL-1α had a correlation with HCV viraemia in this study, the answer can be drawn from both clinical and animal studies

Vanis et al., have reported a significant higher level of IL-1α in patients with chronic HCV compared with healthy subjects **[3]**. In a similar study, Serum level of IL-1α was elevated in patients with Chronic HCV and its related liver diseases (liver cirrhosis and HCC) **[8]**. In animal study, hepatocyte-specific deletion of interleukin receptor type 1(IL-R1) found to attenuate liver injury by blocking IL-1 driving auto-inflammation **[9].** Consequently, we agree with these findings to support the hypothesis that IL-1α as a pro-inflammatory cytokine has a vital role in chronic hepatitis and can reflect the intensity of inflammation and the degree of liver tissue injury.

In the current study, TGF-β1 was significantly higher in all chronic liver diseases than in

healthy subjects. HCC patients recorded the highest level followed by chronic HCV and finally NAFLD groups. These results were corresponding to the cirrhotic features mostly noted in HCC patients.

It was expected to find a significant positive correlation between TGF-β1 and the results of blood fibrosis tests, APRI and FIB-4.This relation is consistent with the recognized role of TGF-β1in hepatic fibrosis and enhances the probability of its use as an indirect indicator of hepatic fibrosis. In fact the role of TGF-β1 in hepatic fibrogenesis in non-viral fibrosis was studied by Raikhelson et al., who found that the expression of TGF-β1 in non-parenchymal liver cells of patients with primary biliary cirrhosis and autoimmune hepatitis with liver cirrhosis was higher than those without liver cirrhosis **[10]**.

Of notice, there is an emerging level of evidence accusing TGF-β1 in pathogenesis of HCC in addition to its role in liver fibrosis. A positive correlation was found between this marker and alpha fetoprotein, indicating a potential synergistic or a causal neoplastic role in patients with chronic liver diseases. Matching with these results, Mehmedovic et al., found that chronic liver disease due to malignant and toxic causes had the highest levels of TGF-β1 followed by chronic viral hepatitis **[11]**. Transforming growth factor β (TGF-β1) plays a dual role in liver carcinogenesis as it inhibits growth of hepatocytes and induces their apoptosis in early stages of tumorigenesis, but once the cells acquire the capacity to overcome its suppressor effects they respond to its underlying epithelial mesenchyme transition which increases their migratory and invasive potential **[12].** In another study, TGF-β1 was proved to have a major role in regulating HCC oxidative metabolism **[13]**. The available level of evidence suggests that TGF-β1 is a promising tool in fighting liver cancer **[14]**.

Our results show a significant negative correlation between IL-1α and TGF-β1 in all studied groups. The results prove their antagonistic roles in chronic liver disease. So the combined measurement of IL-1α as pro-inflammatory marker and TGF-β1 as a fibrosis marker could express the pathological status of the hepatic tissue.

IL-1α was significantly high in all chronic liver diseases than in healthy subjects with no significant difference between groups of liver diseases and each other. Similarly TGF-β1 was significantly high in all hepatic patients than healthy group with the highest significant levels in HCC group.

CONCLUSION

Chronic liver diseases were associated with high levels of IL-1α and TGF-β1 than healthy subjects. IL-1α as a pro-inflammatory cytokine can reflect the intensity of inflammation and the degree of hepatic injury, while TGF-β1 has a major pathogenic part in liver fibrosis and progression to cirrhosis and its level correlates positively with liver fibrosis status. The principal role of TGF-β1 in development of HCC is highly suggestive making it a novel target for HCC management.

Abbreviations;

ECM: Extracellular matrix

TNF-α: tumor necrosis factor alpha

IL-R1: interleukin receptor type 1

TGF-β1: transforming growth factor beta-1

ANA: antinuclear antibody

SMA: smooth muscle antibody

AMA: anti-mitochondrial antibody

NAFLD: Non-Alcoholic Fatty Liver Disease

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Conflict of interest: None.

Ethical consideration:

The study was approved by IRB committee of college of medicine, Zagazig University, Egypt. All procedures were explained to patients and a written or thumb-printed informed consent was obtained.

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