Non Invasive Evaluation of Hepatic Fibrosis in Chronic Hepatitis C Patients

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Abstract— Hepatitis C virus (HCV) is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Hepatic fibrosis is considered the common complication of chronic liver damage. Histological evaluation of liver biopsy specimens had to be the gold standard for the assessment of liver fibrosis, however, it may have some technical limitations and a significant risk. For this reason, a simple, non-invasive reliable biomarker is needed to increase the efficiency of the diagnosis of the patients. We tested tenascin-C (TN-C) in sera of the patients as a simple marker to recognize patients with significant fibrosis from those with non-significant fibrosis. A group of 60 chronic hepatitis C (CHC) patients and 27 healthy controls were tested for their serum TN-C concentrations using enzyme-linked immunosorbent assay (ELISA). Also, liver function tests, routine haematological parameters, Hepatitis markers, Fibroscan and ultrasonography were all estimated. ***Results:*** Serum TN-C levels were significantly higher in CHC patients compared to those of the healthy controls ($P < 0.0001$). In addition, TN-C concentrations in patients with significant fibrosis were statistically different from those of patients with non-significant fibrosis ($P < 0.016$). In addition, TN-C levels were significantly correlated with aspartate aminotransferase activity (AST) ($r = 0.34$, $P < 0.007$), albumin ($r = -0.29$, $P < 0.02$), AST to Platelet Ratio Index (APRI) ($r = 0.33$, $P< 0.01$) as well as Göteborg University Cirrhosis Index (GUCI) ($r = 0.3$, $P< 0.02$). Receiver operating characteristic (ROC) curve of TN-C established to discriminate patients with significant fibrosis from those with non- significant one gave an area under curve (AUC) of 0.73 with a sensitivity of 66.7% and a specificity of 78.8% ($P < 0.001$). ***Conclusion:*** TN-C can be used to assess hepatic fibrosis and evoke significant fibrosis over non-significant one.

Key words — Chronic hepatitis C, Extracellular matix, Liver fibrosis, Tenascin-C

1 INTRODUCTION

During hepatic fibrosis, extracellular matrix (ECM) undergoes continuous remodeling with progressive formation of scar tissue in the form of deposition of ECM proteins in response to hepatocytic injury [1]. Tenascin-C (TN-C) is a hexameric multimodular ECM glycoprotein.[2] Its expression is enhanced during tissue repair. Also, it is intensively induced by inflammation and cancer. It was previously reported that TN-C was accumulated at sites of inflammation, especially at the parenchymal- mesenchymal interfaces [3]. Activation of hepatic stellate cells (HSCs)/Ito cells is the central event in hepatic fibrosis during which such activated cells migrate and localize at sites which necessitate tissue repair. At these sites they not only secrete large amounts of ECM proteins but also cytokines which mediate the process of hepatic fibrogenesis [4]. Elder studies conducted on TN-C-deficient mice indicated the important role of TN-C in progression of liver fibrosis through induction of inflammatory response as well as HSCs activation and recruitment with the simultaneous promotion of collagen type-1 deposition and progressive scar tissue formation. Furthermore, TN-C was found to affect transforming growth factor-β (TGF-β) activation and vice versa [2],[5] For this reason, the present study aims to estimate TN-C levels in sera of patients with chronic hepatitis C to determine whether it can differentiate patients with significant fibrosis from those with non-significant one or not.

2 MATERIALS AND METHODS

2.1 Patients

A total of 60 chronic hepatitis C patients (male, 31; female, 29; mean age, 52 ± 12.6 years) were selected randomly from out patients of Egyptian Liver Research Institute and Hospital (ELRIAH), Dakahlia, Egypt. All patients tested positive for hepatitis C antibodies (HCV-Ab) and were negative for other chronic liver diseases. They had normal kidney function, normal glucose with no liver transplantation. None of the patients had received antiviral treatment before liver fibroscan test and blood sampling. In line, 27 healthy volunteers (male, 14; female, 13; mean age, 33.3 ± 10.15 years) were taken as controls. The controls had normal liver functions and free from any diseases especially liver diseases. Written informed consent was obtained from all the study subjects, and the study protocol was designed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee.

2.2 Biochemical analyses

Samples from all subjects were withdrawn and freshly used. Otherwise, serum samples were kept frozen at -80^{o}C until their use.

2.3 Routine laboratory tests

Complete blood picture (haemoglobin, red blood cells, white blood cells and platelets) were done using D-cell 60 automated hematology analyser (Sysmex X 1800

incorporation, Japan), international normalized ratio (INR) was performed using (Sysmex® CA-1500, Japan) auto analyser, and liver function tests including serum AST, ALT, albumin, bilirubin were done using automated Biochemistry analyser (Cobas Integra 400, Roch, Switzerland).

2.4 Assessment of serum TN-C levels using enzyme-linked immunosorbent assay

Serum levels of TN-C were immunoassayed using sandwich ELISA technique using ELISA kits (Elabscience Biotechnology Co., WuHan, P.R.C.). The optical densities of the developed colors were measured at 450nm and 630nm using (Stat Fax 3200). The corresponding concentrations were calculated from those of the standard curve.

2.5 Serological markers and RT-PCR for detecting HCV infection

Serological markers for detecting HCV infection [hepatitis C antibodies (HCV Abs)] were estimated by ELISA (Merieux anti-HCV, version 4.0, Diasorin S.P.A. via Crescent no 13040 Saluggia (VC) – Italy). HCV RNA was quantized by quantitative RT-PCR using (fully automated Cobas amplified, Taqman48 analyzer, Roch Switzerland).

2.6 Fibroscan and ultrasonography

Liver stiffness [expressed in kilopascals (kPa)] was measured by transient elastography (Fibroscan; Echosens SA, Paris, France). The results obtained were ten valid readings with a success rate of at least 60% and an interquartile range under 30% of the median value. Fibroscan results ranges from 2.5 to 75 kPa. Healthy people without liver disease have a liver scarring reading less than 7.0 kPa (median is 5.3 kPa). A person with chronic hepatitis C and a liver stiffness more than 14 kPa has nearly a 90% probability of having cirrhosis, while patients with liver stiffness more than 7 kPa have around an 85% probability of at least significant fibrosis. Patients were classified according to Fibroscan median into: F0 (no fibrosis, 0-5 kPa), F1 (mild fibrosis without septa, 5.1- 7 kPa), F2 (moderate fibrosis with few septa, 7.1-10 kPa), F3 (severe fibrosis with numerous septa but without cirrhosis, 10.1-17.5 kPa) and F4 (cirrhosis, 17.5-75 kPa). Additionally, the patients were classified into those with non-significant fibrosis (F0-F1) and those with significant fibrosis (F2, F3 and F4).

2.7 Statistical analysis

All statistical analyses were performed by Medcalc software (version 14.8.1.; Medcalc Software Bvba, Ostend, Belgium). Continuous variables were expressed as mean± standard deviation (SD). Comparisons of markers as well as routine laboratory tests and stages of fibrosis were analysed using a two-sided P value. A value of $P < 0.05$ was considered statistically significant. Spearman rank correlation coefficient was used in establishing correlation of TN-C with other parameters and scores. ROC curve analysis was done to determine the cut-off point, area under curve (AUC), Sensitivity, specificity; positive predictive value (PPV) and negative predictive value (NPV) were calculated to define diagnostic accuracy.

3 RESULTS

3.1 Patients' laboratory data

Our study was conducted on 60 CHC patients (male, 31; female, 29; mean age, 52 ± 12.6 years) and 27 healthy volunteers (male, 14; female, 13; mean age, 33.3±10.15 years). According to fibroscan median, patients were classified as 14 patients with F0 (23.3%), 19 patients with F1 (31.7%), 5 patients with F2 (8.3%), 8 patients with F3 (13.3%) and 14 patients with F4 (23.3%).

3.2 TN-C levels

TN-C levels were significantly elevated in sera of CHC patients than in sera of the controls; (86.5 ± 3.14 ng/mL and 58.9 ± 7.23 ng/mL, respectively), ($P < 0.0001$). Serum concentrations of TN-C in sera of patients with significant fibrosis was (87.5 ± 2.9 ng/mL) versus (85.6 ± 3 ng/mL) for patients with non-significant fibrosis. The differences were statistically significant ($P< 0.016$).

3.3 Liver function tests and platelet count

It was found that the mean of activities of ALT and AST were significantly higher in sera of patients with significant fibrosis than in sera of patients with non-significant fibrosis (50.1 ± 20.84 IU/L and 50.1 ± 18.3 IU/L versus 34.75 ± 19.7 IU/L and 30.49 ± 11.1 IU/L, respectively). In addition, the difference in serum albumin was significant when its level in sera of patients with significant fibrosis (4 ± 0.5 gm/dL) was compared with that of patients with non-significant fibrosis (4.49 ± 0.27 gm/dL). The same finding was reported for serum total bilirubin in both groups of patients ($P <0.0001$).

Platelet count was significantly decreased in patients with significant fibrosis (154 ± 73.4 10^9/L) compared to that of patients with non-significant fibrosis (223.42 ± 79 10^9/L), (Table.1).

TABLE 1
THE MEAN VALUES OF TENASCIN-C (TN-C), LIVER FUNCTION TESTS AND PLATELET COUNT OF BOTH NON-SIGNIFICANT AND SIGNIFICANT FIBROSIS PATIENTS WITH THEIR SIGNIFICANCE VALUES

	Control	Non-significant fibrosis (F0, F1)	Significant fibrosis (F2-F4)	*P value
n	27	33	27	
TN-C (ng/mL)	58.9 ± 7.2	85.6 ± 3	87.5±2.9	< 0.016
ALT (IU/L)	25.7 ± 7.5	34.75 ± 19.7	50.1 ± 20.84	= 0.005
AST (IU/L)	26.7 ± 6	30.49 ± 11.1	50.1 ± 18.3	< 0.0001
Alb (g/dL)	4.2 ± 0.7	4.49 ± 0.27	4 ± 0.5	< 0.0001
Bili.T. (mg/dL)	0.4 ± 0.1	0.75 ± 0.073	1 ± 0.12	< 0.0001
Platelet count(10^9/L)	246.19 ± 54	223.42 ± 79	154 ± 73.4	= 0.0002
APRI	----	0.38 ± 0.04	1.17 ± 0.2	< 0.0001
GUCI	----	0.4 ± 0.038	1.4 ± 0.4	< 0.0001
FIB-4	----	1.36 ± 0.14	4 ± 0.9	< 0.0001
King's score	----	8 ± 1	34 ± 8	< 0.0001

*TN-C: Tenascin-C. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. Alb: Albumin. Bili.T: total bilirubin. APRI: AST to Platelet Ratio Index. GUCI: Göteborg University Cirrhosis Index. FIB-4: Fibrosis-4 index. APRI= Normalized AST x 100/platelet countx (109/L), GUCI= Normalized AST × INR × 100/Platelet count(109/l), FIB-4 =age (years) × AST (U/l)/Platelet count (109/l) × ALT (U/l) −1/2 , King's =age × AST (U/l) × INR/Platelet count (109/l). Values are expressed as mean ±SD,as SD is standard deviation, except those of total bilirubin, APRI, GUCI, FIB-4 and king's score expressed as mean± SEM as SEM is standard error of mean. *: P values express significant values when non-significant fibrosis compared to significant fibrosis.*

3.4 Correlation of TN-C with the parameters of liver function tests (LFTs), platelet count, APRI, GUCI, FIB-4 and king's score.

TN-C concentrations showed significant correlations with the numerical values of AST ($P < 0.007$), albumin ($P < 0.02$), APRI ($P< 0.01$) as well as GUCI ($P< 0.02$) . On the other hand, TN-C showed non-significant correlations with all of ALT, total bilirubin, platelet count, FIB-4 and king's score, (Table.2)

3.5 Diagnostic performance of TN-C

Receiver operating characteristic (ROC) of TN-C was performed to differentiate patients with significant fibrosis (F2, F3 and F4) from those with non-significant/minimal fibrosis (F0 and F1).The area under curve (AUC) was 0.73 , the sensitivity was 66.7% and the specificity was 78.8% with a positive predictive value (PPV) of 72% and a negative predictive value (NPV) of 74.3% ($P < 0.01$), (Figure 1 and Table.3).

TABLE 2
CORRELATION OF SERUM TN-C LEVELS WITH OTHER LABORATORY PARAMETERS AND FOUR NON-INVASIVE SCORES

Sample	TN-C	
Parameter	**r**	**P value**
ALT (IU/L)	0.14*	< 0.28
AST (IU/L)	0.34**	< 0.007
Alb (g/dL)	- 0.29**	< 0.02
Bili.T. (mg/dL)	0.153*	< 0.24
Platelet count(10^9/L)	- 0.22*	< 0.09
APRI	0.33**	< 0.01
GUCI	0.3**	< 0.02
FIB-4	0.23*	< 0.08
King's score	0.24*	< 0.06

*P value: P > 0.05 non -significant. P < 0.05: Significant. P < 0.001: More significant. P < 0.0001: Extremely significant. Grade of r: *0.00-0.24 = weak or no correlation; **0.25-0.49 = fair correlation; ***0.5-0.74 = moderate correlation and ****≥0.75 = strong correlation.*

TABLE 3
DIAGNOSTIC POWER OF TN-C IN DIFFERENTIATING PATIENTS WITH SIGNIFICANT FIBROSIS FROM THOSE WITH NON-SIGNIFICANT FIBROSIS

Group	Significant vs non-significant fibrosis						
Parameter	Cut off	AUC	Sn	Sp	PPV	NPV	P value
TN-C	>87.8	0.73	66.7	78.8	72	74.3	< 0.001

*P value: P > 0.05 non -significant. P < 0.05: Significant. P < 0.001: More significant. P < 0.0001: Extremely significant. Grade of r: *0.00-0.24 = weak or no correlation; **0.25-0.49 = fair correlation; ***0.5-0.74 = moderate correlation and ****≥0.75 = strong correlation.*

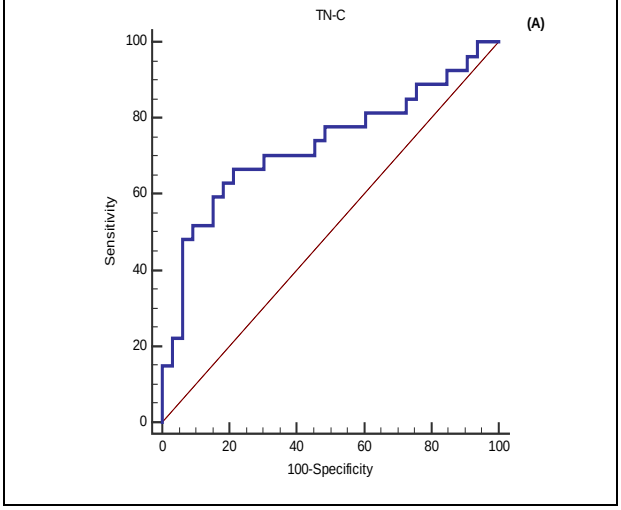

Fig. 1. ROC curve generated by TN-C for differentiating patients with significant fibrosis from those with non-significant fibrosis; (AUC) = 0.73.

4 Discussion

Continuous untreated hepatocytic damage develops into chronic liver disease that is characterized by prominent inflammatory infiltrate with progressive deposition of ECM proteins secreted by HSCs which are the main ECM-producing cells in the injured liver [6],[7]. It was previously elucidated that TN-C expression is commonly upregulated with the ongoing inflammatory conditions. This may be the case in the present study and this is because TN-C levels were significantly higher in patients with significant fibrosis than those of patients with in non-significant one. Further, it enhances the motility and migration of lymphocytes by stimulating their tethering and rolling and by retarding their attachment to ECM [8]. Thus, one can suggest that TN-C renders the involvement of immune system in hepatic fibrogenesis which is usually the case. In the same line, TN-C also stimulates the secretion of inflammatory cytokines such as tumor necrosis factor α (TNF-α), interferon γ (IFN-γ) and interleukin 4 (IL-4) in many experimental models [2],[9],[10],[11]. Again, the latter cytokines stimulate HSCs to secrete more ECM proteins and exaggerate hepatic fibrosis. A recent study by Jian kang et.al [12] reported that recombinant tenascin-C (rTN-C) promoted HSCs migration with upregulation of collagen type-I expression via TGF-β1 and α9β1 integrin signaling pathways. This confirms our suggestion in that TN-C modulate liver fibrogenesis through an immune system-mediated mechanism.

The significant and positive correlations between TN-C and both APRI and GUCI scores confirm the interrelationship between TN-C and the severity of the disease.

5 Conclusion

TN-C not only participate in hepatic fibrosis through an immune mediated mechanism but also correlates with the severity of the disease. For this reason, TN-C arises to be a molecular target in therapeutic strategies to attenuate liver fibrosis.

6 References

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