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YKL-40 As a Non Invasive Predictor of Liver Fibrosis After Combined Interferon /Ribavirin Therapy in Chronic Hepatitis C infection

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Abstract: Liver fibrosis and cirrhosis represent the main consequence of chronic liver diseases of different origin. Therefore, the clinical assessment of disease severity is a must in the management of patients with chronic liver damage. Several non-invasive tests for the assessment of disease severity have been proposed, among these numerous serum markers, hyaluronic acid (HA) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), have been the most extensively studied, especially in patients with chronic hepatitis C (CHC). This study aimed to evaluate the associations between pre -treatment serum levels of HA, TIMP-1 and YKL-40 and disease severity after treatment in patients with CHC. A total of 80 patients with HCV-associated liver disease were enrolled. Serum HA, TIMP-1 and YKL-40 levels were measured. The patients received combined peginterferon and ribavirin therapy. The relationship between the concentrations of serum fibrosis markers and Ishak fibrosis scores and evaluated the changes of the levels of fibrosis markers before and after the IFN therapy. After 48 weeks of combined peg-IFN/RBV, about 51 patients (63.75%) were achieved sustained viral response (SVR group) and 29 (36.25%) patients were failed to response to therapy after 12, or 24 week of starting therapy. The serum fibrosis markers were significantly decreased in SVR group as compared toits pre-treatment levels. In non responder group, the level of YKL-40 was significantly decreased, after therapy, while the levels of HA and TIMP-1 were not statistically different. The increase in serum levels of all markers, particularly YKL-40 and HA were correlated with the progression of liver fibrosis and only YKL-40 was correlated with grade of necro-inflammation. Conclusion: YKL-40 may be a useful non-invasive serum marker to estimate the degree of liver fibrosis and pre-treatment YKL-40 levels are an independent predictor of initial virological response to peginterferon and ribavirin treatment. Levels of all three serum fibrosis markers decreased significantly in the SVR patients, consistent with reduced hepatic fibrogenesis. Measuring serum fibrosis marker levels before and after antiviral therapy may provide important prognostic information in CHC patients.

Key words: Liver fibrosis · Chronic Hepatitis C (CHC) · Ykl 40

INTRODUCTION

Liver fibrosis is a dynamic process characterized by a balance of extracellular matrix deposition and degradation; it represents the response of the liver to different chronic insults and is associated with significant morbidity and mortality [1]. The severity of hepatic fibrosis and inflammation at diagnosis correlates strongly with the likelihood of disease progression in patients with chronic hepatitis C (CHC) [2]. In addition, histological staging in patients with CHC influences disease monitoring as well as antiviral treatment decisions [3, 4]. Despite improvements in the efficacy of treatment of chronic hepatitis C, therapy is not recommended for all patients because of the indolent course in some patients and the expense, unpleasant side effects and low overall efficacy (50% to 60%) of current treatment. Thus, accurate determination of the extent of hepatic fibrosis or stage of liver disease is important for prognosis, decisions regarding treatment and monitoring

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of disease progression [5, 6]. Administration of 'maintenance' interferon to patients with CHC who failed to respond to a course of antiviral treatment may slow the rate of liver disease progression [7]. Several large randomised controlled studies were initiated in prior non-responders to determine if maintenance interferon could reduce the rate of clinical decompensation and histological disease progression [8, 9].

Although liver biopsy may be associated with sampling error, inter- observer variability and potential complications, it still remains the gold standard for establishing the severity of hepatic necro-inflammation and fibrosis [10]. Therefore, accurate and reliable non-invasive means to assess patients with chronic hepatitis C (CHC) at increased risk of developing worsening hepatic fibrosis are needed [11]. Among the numerous proposed serum markers, serum hyaluronic acid (HA) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), have been the most extensively studied, especially in patients with CHC [12]. In these patients, a significant correlation was found between serum HA and TIMP-1 levels and the degree of hepatic fibrosis. However, the accuracy of these serum markers in diagnosing cirrhosis is extremely low [13]. Recently, YKL-40, a new protein expressed in human liver. YKL-40 is a fibroblast and endothelial growth factor that belongs to the chitinase family with a molecular weight of 40 kd. The physiologic functions of YKL-40 are unknown. YKL-40 is produced in a wide variety of cell types, including chondrocytes, synovial cells [14], activated macrophages [15], neutrophils [16] and in particular from cells located in tissues with increased remodeling/degradation or inflammation of the extracellular matrix, such as the hepatic stellate cells [17]. Serum YKL-40 has been reported to be superior to hyaluronic acid levels in predicting hepatic fibrosis [18]. A recent study of 129 patients with different causes of chronic liver disease found that serum YKL-40 levels were increased, even in patients with mild fibrosis and correlated better with histological fibrosis scores than serum hyaluronic acid levels [17, 19]. Further studies in larger numbers of patients are needed to confirm the utility of testing for YKL-40 levels in assessing hepatic fibrosis in patients with CHC.

Aim of the Study: This study aimed to evaluate the base line serum levels of hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and YKL-40 levels in CHC patients and their associations with disease outcome after combined peg-IFN/RBV therapy.

Patients and Methods: The present study conducted on 80 patients (59 males and 21 females) with chronic HCV infection diagnosed by the presence of HCV RNA and histopathological features. The patients were recruited from National Liver Institute and were underwent for combined interferon (IFN) and ribavirin therapy after fulfilment of the inclusion criteria (Naive CHC infected patients HCV-genotype 4, negative both HBs Ag and HBe Ag, age >18 years, persistently (>6 months) elevated liver enzymes 1.5 fold above upper limit of normal and fibrosis stage from 1-4 on Ishak score, CBC: normal WBCs and platelets >100.000/ mm^3), PT < 2 seconds increase above upper normal range, albumin > 3.5gm/dl, normal renal function tests and controlled blood glucose level in diabetic patients, TSH within normal range and ANA titre <1: 20 and no evidence of decompensated cirrhosis. The study was approved by the Ethical Committee of National Liver Institute, Menoufiya University and a written informed consent was obtained from all participated subjects.

All Participants Were Subjected to the Followings:

- Pre-treatment evaluation including: Full history taking and full clinical examination, BMI calculation, abdominal ultrasonography, ultrasound-guided liver biopsy to assess the stage of fibrosis (1-6) and grade of necro-inflammation (1-18) according to the Ishak scoring system [20].
- The following laboratory investigations were done: CBC using Sysmex K-21 (Japan), prothrombin time and concentration using Fibrintimer (Dade Behring Diagnostic Inc, Germany) liver and renal function tests using auto Analyzer Cobas Integra-400 (Roche-Germany). Hepatitis markers: HBs Ag and HCV Ab were assayed by EIA (COBAS-core, Germany).
- Quantitative HCV RNA levels were performed by real-time PCR at baseline, weeks 12, 24 and 48 during treatment to determine virological response. HCV RNA and HCV genotyping was measured by Cobas Amplicor HCV monitor-version-2 using a commercial kit from (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's instructions.
- Serum samples were tested by a commercially available quantitative ELISA for HA (HA-ELISA; Echelon Biosciences Inc., Salt Lake City, UT, USA) and TIMP-1 (Quantikine, R and D Systems, Minneapolis, Minnesota, USA), according to the manufacturer's instructions.

- YKL-40 was measured using commercially available bioassays (Metra, Biosystems, San Diego, California, USA), according to the manufacturer's instructions.

The standard care of treatment protocol consisted of combination of pegylated interferon (PEG-IFN) α2a or α2b in addition to ribavirin (RBV) for up to 48 weeks. Those completed 48 weeks (SVR) were followed up for 24 weeks after the end of therapy. Patients with an insufficient virological response at 12 or 24 weeks (detectable HCV RNA) were considered to have treatment failure (non responders) and therapy was discontinued.

Patients follow up: Clinical follow up to report and manage any possible adverse effects of therapy and laboratory assessment for CBC, liver and renal function tests and viral load to assess treatment response. Serum HA, TIMP-1 and YKL-40 were also measured at the end of therapy (week 48 for non responder) and (week 24 for non responders).

Statistical Analysis: Data were expressed as mean ± SD. The SPSS computer program version 12.0 was used for statistical analysis. Student's t-test for parametric data and the Kruskal-Wallis multiple comparison Z-value test for nonparametric data were used. Chi square (X^2) was used to compare qualitative variables. All tests were two-tailed and p-values <0.05 were considered significant.

RESULTS

Table 1 represent the demographic data and Table 2 represent the laboratory base line data of the studied patients before therapy, where the mean levels of HA was 269±73 ng/ml, TIMP-1 was 958±295 ng/ml and YKL-40 was 138±41138±41 μg/L. After 48 weeks of combined peg-IFN/RBV, about 51 patients (63.75%) were achieved sustained viral response (SVR group) and 29 (36.25%) patients were failed to respond to therapy after 12, or 24 week of starting therapy. The serum fibrosis markers were significantly decreased in SVR group as compared to its pre-treatment levels (p<0.01; <0.001, <0.0001) for HA, TIMP-1 and YKL-40 respectively (Table 3 & Fig. 1). In the non responder group, although the level of YKL-40 was significantly decreased (p<0.05), after therapy, the levels of HA and TIMP-1 were not statistically different (Table 3 & Fig. 2). As regard the correlation study of

Table 1: Demographic data of CHC patients at base line before therapy

Variables	Chronic HCV patients (N=80)
Age (Years) (M±SD)	41.7 ± 8.6
Male gender	59 (73.8%)
Hepatomegaly	52 (65%)
Splenomegaly	26 (32.5%)
BMI (kg/m^2) (M±SD)	24.1±3.8
Fibrosis stage:	
Stage 1	21 (26.3%)
Stage 2	30 (37.5%)
Stage 3	16 (20%)
Stage 4	13 (16.2%)
Necro-inflammation	
Grades 1–5	25 (31.2%)
Grades 6–8	43 (53.8%)
Grades >8	12 (15%)

Table 2: Laboratory data of CHC patients at base line before therapy

Parameters	Chronic HCV patients (n=80)
ALT (U/L)	93.6 ±28.2
AST (U/L)	78.5 ±21.4
GGT (U/L)	41.2±7.1
S. albumin (gm/dl)	3.8 ± 0.25
Prothrombin conc. %	76.3±5.4
WBCs (x10^3/μl)	5.9±1.7
Hemoglobin (gm/dl)	12.2 ±1.6
Platelet counts (x10^3/μl)	201±95
Viral load (log IU/mL)	8.7± 3.1
HA (ng/ml)	269±73
TIMP-1 (ng/ml)	958±295
YKL-40 (μg/L)	138±41

Table 3: Serum fibrosis markers in of CHC patients before and after therapy

	Sustained responders (SVR) (N=51)			Non responders (NR) (N=29)		
Parameters	Base line	End of follow up	p-value	Base line	End of follow up	p-value
HA (ng/ml)	185±102	109±64	<0.01**	295±93	262±79	>0.05
TIMP-1 (ng/ml)	823±272	559±342	<0.001**	857±136	922±258	>0.05
YKL-40 (μg/L)	123±46	87±21	<0.0001**	152±39	117±28	<0.05*

*p value <0.05 is statistically significant, **p value <0.001 is statistically highly significant & p value >0.5 is non significant.

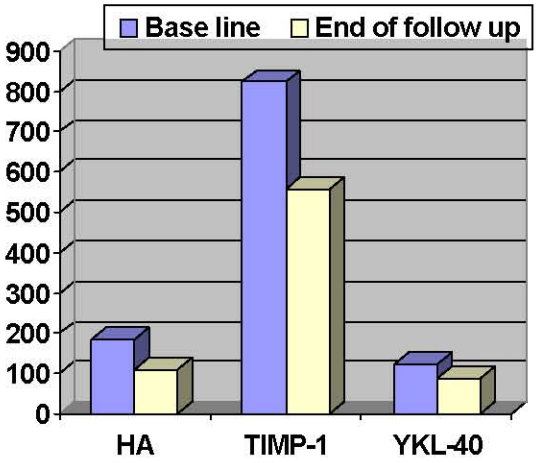

Fig. 1: Serum levels of fibrosis markers in SVR at base line and end of therapy

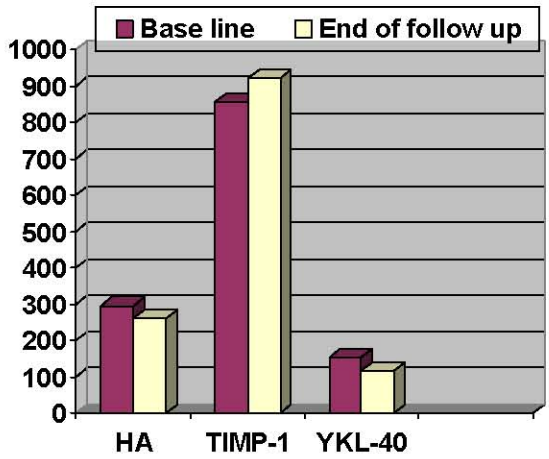

Fig. 2: Serum levels of fibrosis markers in NR at base line and end of therapy

Table 4: Correlation of serum fibrosis markers with prognostic parameters in CHC patients

Parameters	HA	TIMP-1	YKL-40
ALT (U/L)	R = 0.13	r = 0.07	r = 0.11
	p>0.05	p>0.05	p>0.05
S. albumin (gm/dl)	r = -0.46	r = -0.53	r = -0.57
	p<0.05*	p<0.01**	p<0.01**
Prothrombin conc. %	r = -0.51*	r = -0.45	r = -0.61
	p<0.05	p<0.05*	p<0.01**
Platelet counts (x10^3/µl)	r = -51*	r = -21	r = -64
	p<0.05*	p>0.05	p<0.00**
Fibrosis stage	r = 0.49	r = 0.57	r = 0.68
	p<0.05*	p<0.01**	p<0.001**
Necro-inflammation	r = 0.13	r= 0.22	r = 0.47
	p>0.05	p>0.05	p<0.05*
HA (ng/ml)	--	r = 0.42	r = 0.73
		P<0.05*	P<0.001**

serum fibrosis markers with prognostic parameters in CHC patients , YKL-40 level was negatively correlated with S. albumin, prothrombin concentration, platelet counts and strongly correlated with grade of necro-inflammation, stage of liver fibrosis and HA serum level (Table 4). HA and TIMP-1 were negatively correlated with S. albumin, prothrombin concentration and positively correlated with stage of liver fibrosis and not correlated with necro-inflammation (Table 4).

DISCUSSION

Liver biopsy is currently the gold standard for the diagnosis of liver fibrosis, but it is an invasive procedure with associated morbidity that carries a significant cost. For these reasons, a reliable non-invasive fibrosis marker is required but a reliable and less invasive serologic diagnostic tool is needed [18]. In the previous studies, simple non-invasive methods without biopsy to predict both significant fibrosis and cirrhosis have been investigated. Measurements of mixed parameters, such as the blood test [21], fibrotest [22] or aspartate aminotransferase (AST) to platelet ratio [23] indices (APRI), have been assessed as substitutes for liver biopsy, but these methods are difficult to be calculated, do not reflect the mechanism of the liver fibrosis directly and can not predict the efficacy of IFN treatment in patients with HCV-associated liver disease. Therefore, our study was designed to find the role of a recent non invasive fibrosis marker; YKL-40 in assessing disease progression in 80 CHC patients after combined peg-IFN/RBV therapy compared to conventional markers, HA and TIMP-1. After 48 weeks of combined peg-IFN/RBV, about 51 patients (63.75%) achieved sustained viral response (SVR group) and 29 (36.25%) patients failed to respond to therapy after 12, or 24 weeks of starting therapy. In our study, we also examined which fibrosis marker reflected the response to IFN therapy in those patients. The serum fibrosis markers were significantly decreased in SVR group as compared to its pre-treatment levels ($p<0.01$; <0.001, <0.0001) for HA, TIMP-1 and YKL-40 respectively. In the non responder group, although the level of YKL-40 was significantly decreased ($p<0.05$), after therapy, the levels of HA and TIMP-1 were not statistically different. Saitou *et al.* [24] and Prado *et al.* [25] reported that, the levels of HA and YKL-40 significantly decreased after IFN treatment. The YKL-40 levels lowered significantly not only in the SVR group, but also in the non responders group after IFN therapy.

Previous studies had linked serum HA, TIMP-1 and YKL-40 levels to disease severity and its progression in patients with CHC [24, 26]. In particular, these serum fibrosis markers were reduced in SVR compared to relapsers and non responders, suggesting that they are closely linked with hepatic fibrogenesis [24, 27]. In addition, previous longitudinal studies have linked serum and liver tissue expression of YKL-40 with the risk of fibrosis progression [28]. The use of scores including direct fibrogenesis markers may be an advantage in certain situations, such as in patients with CHC who undergo antiviral therapy. Since the markers included in such scores are directly related to the fibrogenesis process, their assessment might be useful to monitor liver fibrosis regression, a well documented finding in individuals achieving a sustained virological response [29, 30]. In previous studies, significant declines from baseline of PIIIP and YKL-40 were also noted at week 72 in patients with an SVR compared with non responders [27].

The identification of baseline YKL-40 levels as an independent predictor of clinical outcomes is a novel and potentially important finding. In addition, serum HA and TIMP-1 levels also were significantly increased over time and were consistently higher in patients who clinically progressed compared with patients who did not [31]. These data suggest that serial assessment of these markers may prove useful to the clinicians in the follow up of patients with CHC. However, a previous study had also demonstrated a non-specific increase in PIIINP and HA levels in patients receiving full-dose peginterferon and ribavirin treatment [27]. Therefore, the impact of low-dose peginterferon on serum fibrosis marker levels was explored. In Martinez *et al.* [32] study, the significant increase in serum TIMP-1 levels observed at the end of follow up in non-sustained virological responders may indicate that fibrosis is progressing in these patients. Indeed, other reports found a similar TIMP-1 increase following interferon alfa therapy in non responder patients [33, 34]. TIMP-1 protects collagen from fibrolysis by the matrix metalloproteinases and also inhibits the apoptosis of hepatic stellate cells [35]. In experimental models, over expression of TIMP-1 was associated to enhanced fibrosis, supporting the hypothesis that inhibition of matrix degradation may contribute to the progression of fibrosis [36].

Previous studies have shown that HA levels reflect an increased production of this marker by hepatic stellate cells as well as a decreased removal from circulation, which depends on the uptake by specific receptors in hepatic sinusoidal endothelial cells [36]. Higher HA levels and lower probability of virological response could reflect dysfunction of endothelial sinusoidal cells that is present in patients with more advanced liver fibrosis, another independent predictor of virological response [32].

The short term effects of IFN therapy on histological improvement are well documented and most studies have shown histological improvement at the end of treatment and/or within 1-2 years of follow-up [37], even though hepatitis C virus was not completely eradicated. IFN treatment also reduced activity in the SVR group and even in non responders patients at 6-12 months after completion of IFN treatment [24], suggesting the anti-inflammatory effects of treatment in these patients.YKL-40 levels were strongly correlated with histological markers of stellate cells activation and the risk of rapid fibrosis progression in liver allograft recipients with recurrent hepatitis C [38]. Furthermore, functional polymorphisms in YKL-40 gene expression are associated with the severity of liver fibrosis in patients with CHC, further strengthening the potential importance of this protein in hepatic fibrogenesis [39]. Therefore, Fontana *et al.* [31] data added to the growing body of literature supporting the potential utility of YKL-40 as a clinically useful biomarker for hepatic fibrosis. Interestingly, in this study, these serum fibrosis markers were correlated strongly with patients outcome in CHC patients, YKL-40 level was negatively correlated with S. albumin, prothrombin concentration, platelet counts and strongly correlated with grade of necro-inflammation, stage of liver fibrosis and HA serum level (Table 4). HA and TIMP-1 was significantly negatively correlated with S. albumin, prothrombin concentration and positively correlated with stage of liver fibrosis and not correlated with necro-inflammation. This finding was consistent with Fontana *et al.* [31] who reported that, HA, TIMP-1 and YKL-40 levels are all associated with clinical progression when combined with baseline albumin and INR levels individually. Also, pervious studies [40, 41] demonstrated the short-term prognostic utility of markers of liver synthetic function (albumin and INR) and excretory function (bilirubin) in patients with CHC and advanced fibrosis. However, Fontana *et al.* [31] demonstrated that the addition of baseline HA, TIMP-1 or YKL-40 levels to these routine laboratory parameters provided important incremental ability to predict clinical outcomes in patients with CHC [42, 43]. Nojgaard *et al.* [44] reported that serum levels of YKL-40 represented ongoing fibrosis like HA, in addition to fibrogenesis similar to type IV collagen and PIIIP of the liver disease. Saitou *et al.* [24] reported that

serum YKL-40 levels were valuable for diagnosing mild stage of fibrosis at a cut-off value <186 ng/ml, severe stage of fibrosis at <284 value and cirrhosis at <284.8 value in HCV-associated liver disease. These results suggested that YKL-40 might be a new useful marker for monitoring liver fibrosis. Saitou *et al.* [24] reported that, YKL-40 and HA were found more useful than other markers for assessing the fibrosis stage. In particular, YKL-40 was most useful for monitoring the fibrosis of liver disease and for distinguishing extensive liver fibrosis from mild stage of liver fibrosis and HA appeared to be slightly better for prediction of cirrhosis from chronic hepatitis than YKL-40.

Baseline levels of YKL-40, PIIINP, TIMP-1 and HA were all associated with the risk of fibrosis progression [31]. However, a baseline HA and platelet counts was most strongly associated with histological progression. Low platelet counts are well known to be associated with more severe hepatic fibrosis, a reflection of portal hypertension and hypersplenism as well as reduced thrombopoietin production [23, 45]. Similarly, baseline and serial platelet levels were reported to be associated with a higher likelihood of developing varices and death in patients with alcoholic cirrhosis [46]. The increase in serum HA levels associated with worsening hepatic fibrosis has been attributed to the reduced HA clearance by hepatic sinusoids and increased HA production by hepatic stellate cells [47]. Plasma levels of this glycosaminoglycan, which is synthesized by HSC and degraded by liver sinusoidal cells, are increased in CHC due to delayed degradation and is correlated with the stage of fibrosis [48]. Its main value is to exclude advanced fibrosis or cirrhosis; however, it loose much of its usefulness in distinguishing among earlier stages of fibrosis. YKL-40 has shown good specificity (81%) and sensitivity (78%) for distinguishing between mild and significant fibrosis in CHC [24]. HA is a polysaccharide found in virtually all connective tissues and in liver fibrosis [49], it is a component of the extracellular matrix [50]. In chronic hepatitis, HA is synthesized by the hepatic stellate cells and is metabolized in the liver endothelial cells [47]. With severe fibrosis in chronic hepatitis, increasing deposition of basement membrane components causes sinusoidal capillarization, diminishing HA clearance. HA levels increase, particularly in patients with cirrhosis [13]. Therefore, it is thought that HA levels reflect fibrosis. In another study in Europe, a panel of 10 markers were evaluated; PIIINP, HA, TIMP-1 and collagen IV correlated best with histological fibrosis scores [51].

CONCLUSION

These results suggest that, serum markers of fibrosis could replace liver biopsy in monitoring the progression of fibrosis in patients with CHC.YKL-40 might promptly reflect the improvement of liver inflammation, in SVR group after IFN treatment. Therefore, the serum level of YKL-40 changes might reflect the efficacy of the IFN treatment in CHC patients more directly and dynamically than other fibrosis markers. In addition, YKL-40 might estimate the therapeutic effect before IFN treatment, since YKL-40 value was relatively lower in SVR group than that in non responder group. Further studies with large cohort of patients are needed to confirm the potential usefulness of non invasive markers and to correlate these markers with ultrasonographic findings and liver histology.

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