Original Article

THAI J GASTROENTEROL 2014
Vol. 15 No. 1
Jan. - Apr. 2014
15

Six Month Follow-up of Liver Stiffness Measurement in Untreated Chronic Hepatitis C With or Without HIV Co-infection

Phrommee N
Leerapun A
Pisespongsa P
Chitapanarux T
Kitdumrongthum P
Praisontarangkul O
Thongsawat S

ABSTRACT

Objectives: There is little data on monitoring liver fibrosis progression using the new noninvasive "transient elastography" method, especially in untreated HCV patients. To address this issue, the prospective cohort study was designed to assess transient elastography changes after a 6-month interval, and to compare data between the HCV mono-infection group and the HCV/HIV co-infection group.

Method: Untreated HCV adult patients with or without HIV co-infection attending our clinic between November 2011 and March 2012 were enrolled. Liver stiffness (LS) measurement by transient elastography method (Fibroscan®) was performed at baseline and reported after 6-month. Fibrosis staging followed the METAVIR system with cut-off LS level >7.1 kPa for F2, >9.5 kPa for F3 and >12.5 kPa for F4. The primary end-point was fibrosis progression in both groups.

Results: One-hundred-and-thirty-one patients were recruited between November 2011 and March 2012. A total of 129 patients performed the baseline demographic data analysis, and 115 patients in the pair analysis. The 73 patients (56%) had HCV mono-infection, and 56 patients (44%) had HCV/HIV co-infection. More advanced age (49.9 vs. 42.3 years, $p<0.001$) and advanced liver disease (F4, LS>12.5kPa, 48% vs. 16.1%, $p<0.001$) were observed in the HCV mono-infection group. Modest increase of LS at 6-month was seen in both groups, but not statistically different (LS change 3.36 kPa and 1.54 kPa, $p=0.107$, for the HCV and the HCV/HIV group respectively). During the study, there were 2 of 4 patients died from liver-related disease.

Conclusion: In first 6-month interval of our study, fibrosis progression was demonstrated by using LSM, however, no difference between the both groups. Long term study should be continuing.

Keywords: Liver stiffness measurement (LSM), liver fibrosis, HCV infection, HCV/HIV co-infection

[Thai J Gastroenterol 2014; 15(1):15-21.]

Division of Gastroenterology, Department of Internal medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Address for Correspondence: *Apinya Leerapun, M.D., Division of Gastroenterology, Department of Internal medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.*

INTRODUCTION

Chronic hepatitis C (CHC) infection is the leading cause of liver cirrhosis and hepatocellular carcinoma, and is the main indication for liver transplantation in the Western world especially in the USA[1] around, 15-30% progress to cirrhosis over three decades after diagnosis[2]. Many factors including HIV co-infection are associated with an increased rate of fibrosis development, causing an exponential natural history to develop cirrhosis[3]. HCV and HIV viruses share the same route of transmission. Liver disease is the second leading cause of death in HIV-infected patients in the HAART era[4]. Many studies utilizing paired liver biopsy reported more rapid fibrosis progression in HCV/HIV co-infected patients than in HCV mono-infected subjects. But the low acceptable rate and long interval time (more than two or three years) for repeated this procedure were observed[5-6]. In recent years, there are newer noninvasive methods to evaluate liver fibrosis in chronic viral hepatitis, mainly in hepatitis C. Liver stiffness measurement (LSM) by transient elastography is a promising method that has been well validated with high diagnostic accuracy for advanced fibrosis/cirrhosis as well as good reproducibility[7]. The primary end-point in our study was to evaluate the rate of fibrosis progression in untreated CHC, using LSM at 6-month interval in both HCV and HCV/HIV co-infected groups.

METHODOLOGY

Study population

Anti-HCV positive patients aged 18 and over who we and regularly followed-up at the Gastrointestinal / Hepatology clinic and the HIV clinic, Maharaj Chiang Mai University Hospital, were recruited. Participant gave a written informed consent and answered baseline questionnaires including the probable route and the year of HCV acquisition. The estimated duration of infection was calculated. Exclusion criteria were previous HCV treatment, presence of HCC, jaundice, ascites, and congestive heart disease or valvular heart disease. Liver stiffness measurement (LSM)

Participants underwent liver stiffness measurement using transient elastography method (Fibroscan®) within 1 month after enrollment, and again at 6-month thereafter. LSM was performed with the patient lying in dorsal decubitus with the right arm in maximal abduction. The probe was placed on the right lobe of the liver, in-between intercostal spaces. The measurement depth was between 25 and 65 mm using the M probe of FibroScan®. Ten validated measurements were taken at each procedure. Results were expressed in kilopascals (kPa). Only procedures with at least 10 validated measurements and an interquartile range < 30% of the median value were considered reliable. Measurement of liver stiffness in our study was performed by one investigator. Patients had nothing per oral (NPO) at least 4 hours before the procedure. Measurement data were classified into 4 groups; LS, cut-off > 7.1 kPa for F2, > 9.5 kPa for F3 and > 12.5 kPa for F4[8].

Laboratory data

Data on CBC, BUN, Cr, LFT, PT, INR within preceding 3 months, and FBS, CBC, CD4 count within preceding 6 months, as well as HCV viral load and HIV viral load within preceding 1 year before each LSM were collected. If any such data were not available, the missing test blood drawing for was done on the date of LSM.

Statistical analysis

Statistical analysis was performed using SPSS statistical package, version 17.0. Descriptive statistics were performed using Chi-square test and two-way ANOVA (categorical data), Paired student *t*-test or Mann Whitney U test (continuous data). Multivariate binary logistic analysis using robust standard error was performed to find factor of the occurrence of any definite related adverse events. A two-side p value <0.05 was considered to be significant.

This study was sponsored mainly by the Research Institute of Health Science (RIHES), Chiang Mai University. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University.

RESULTS

Demographic data

One-hundred-and-thirty-one patients were recruited between November 2011 and March 2012, 125 of whom underwent first LSM. During the 6-month interval, 4 patients were died, 1 received HCV treatment, and 5 were lost to follow-up. The remaining 121 participants underwent a repeat LSM 6 month after the first test, thus a total 115 completing two LSM procedures.

Two patients were excluded because of unsuccessful LSM, both with HCV mono-infection, hence a total of 129 patients in the baseline demographic data analysis, and 115 patients in the pair analysis (64 patients, 56% with HCV mono-infection).

Of 129 patients, 73 (56%) had HCV mono-infection, and 56 (44%) had HCV/HIV co-infection. Male was predominant in both groups, 45 (63%) and 32 (57%). The mean age in the HCV mono-infection group was significantly higher than in the HCV/HIV co-infection group (49.9 vs. 42.3 years, $p<0.001$), as were BMI and waist circumference. More patients in the HCV mono-infection group had history of alcohol intake (Table 1).

Regarding the route of HCV acquisition, history of transfusion of blood product was significantly more frequent in HCV mono-infected patients (48.6% vs. 30.4%, $p=0.049$). On the other hand, history of body piercing and homosexuality (MSM) was significantly more common in the HCV/HIV co-infection group, history of IVDU and tattooing appeared to be frequent. The duration of HCV infection was longer in the HCV mono-infection group (30.30 vs. 24.46 years, $p=0.044$), with most subjects being infected more than 20 years (Table 1).

Although serum aminotransferase levels were

Table 1. Baseline demographic data.

	HCV mono-infection	HCV/HIV co-infection	*p*-value
Number of patients (n, %)	73 (56)	56 (44)	
Age (yrs)	49.92 (10.07)	42.30 (7.37)	<0.001
Male (n,%)	4 5(61)	32 (57)	0.74
Route of HCV acquisition (n, %)			
IVDU	10 (13.5)	11 (25)	0.115
Tattoo	25 (33.8)	16 (36.4)	0.776
Piercing	23 (31.5)	26 (54.2)	0.013
Blood transfusion	36 (48.6)	14 (30.4)	0.049
Homosexual (MSM)	0	5 (8.9)	0.034
Duration of HCV infection (yrs)*, mean	30.36 (13.05)	24.46 (7.55)	0.003
Group of duration of HCV infection, yrs			0.003
0 - 5	3 (4.3)	0 (0)	
6 -10	4 (5.7)	1 (2.6)	
11 - 15	2 (2.9)	2 (5.1)	
16-20	6 (8.6)	14 (35.9)	
> 20	55 (78.6)	22 (56.4)	
BMI (Kg/m^2)	24.02 (4.02)	21.73 (3.23)	0.001
Waist circumference	83.04 (12.15)	78.81 (9.20)	0.032
Alcohol comsumption (n, %)			0.018
Yes	13 (17.3)	21 (37.5)	
Stop > 6 month	49 (65.3)	24 (42.9)	
No	13 (17.3)	11 (19.6)	
Smoking (n, %)			0.858
Yes	13 (17.6)	12 (21.4)	
stop > 6 month	21 (28.4)	15 (26.8)	
No	40 (54.1)	29 (51.8)	
HCV viral load (x 106 IU/mL), mean**	2.40 (2.34)	1.46 (2.70)	0.028
< 400,000 (n, %)	16 (23%)	40 (76%)	<0.001
> 400,000 (n, %)	54 (77%)	13 (24%)	
Undetectable HCV viral load (n, %)**	3 (4.3)	33 (62.3)	<0.001

Table 1. Baseline demographic data. (cont.)

	HCV mono-infection	HCV/HIV co-infection	*p*-value
CD_4 count [CD_4%], median	-	448 (18%)	NA
HIV viral load (copies/mL), median	-	<20	NA
Liver stiffness (kPa), mean	17.11 (13.49)	9.04 (9.07)	<0.001
Liver stiffness group			
F0-1 <7.1 kPa	16 (22.9)	37 (66.1)	<0.001
F2 7.1-9.4 kPa	13 (18.6)	9 (16.1)	
F3 9.5 - 12.4 kPa	7 (10.0)	1 (1.8)	
F4 >12.5 kPa	34 (48.6)	9 (16.1)	
Hemoglobin (mg/dL)	13.87 (1.79)	13.76 (1.79)	0.731
Hemotocrit (%)	41.79 (4.69)	41.22 (4.73)	0.493
WBC count	5,785.47 (1768.92)	7,592.5 (8510.81)	0.103
% Neutrophils	53.83 (9.78)	51.98 (10.01)	0.292
% Lymphocyte	33.28 (9.37)	33.02 (8.63)	0.873
Platelet counts	158,533.33 (67248.5)	227,482.14 (83830.35)	<0.001
Albumin	3.91 (0.72)	4.26 (0.59)	0.005
Globulin	5.94 (10.45)	3.78 (0.8)	0.001
ALP	86.72 (41.11)	90.91 (31.17)	0.127
AST	92.42 (76.99)	48.71 (41.92)	<0.001
ALT	93.27 (68.36)	49.66 (41.15)	<0.001
TB (mg/dL)	2.35 (9.90)	0.7 (0.46)	<0.001
DB (mg/dL)	0.32 (0.45)	0.15 (0.18)	<0.001
PT (sec.)	11.16 (1.47)	11.85 (12.55)	<0.001
INR	1.04 (0.12)	0.97 (0.09)	<0.001
FBS	95.14 (21.77)	94.17 (15.87)	0.782
TG	103.52 (42.71)	162.7 (97.72)	<0.001
HDL-chol	44.07 (14.04)	49.12 (13.46)	0.041
VLDL-chol	20.93 (8.56)	32.8 (19.54)	<0.001
LDL-chol	107.77 (34.8)	103.88 (31.59)	0.514

All present in mean (SD)

*data from 107 patients (68/73, 93% in HCV and 39/56, 70% in HCV/HIV)

**data from 123 cases [a] include 1 was bisexual

higher in the HCV mono-infection group, multivariate analysis showed only age being significantly higher in the HCV mono-infection group.

Liver stiffness (LS) result

At baseline, the mean LS was higher in the HCV mono-infection group (17.11 and 9.04, $p<0.001$). Based on the METAVIR system, most patients in this group were F4 (48% vs. 16.1%, $p<0.001$), in contrast with patients in the HCV/HIV co-infection group who were mostly F0-1 (66.1% vs. 22.7%, $p<0.001$) (Table 1). At 6 months later, the distribution of LS remained the same.

About two-thirds of all patients (78/115 or 67.8%) had increased LS (45 HCV and 33 HCV/HIV). The change was modest (5.95 kPa in the HCV group and 3.49 kPa in the HCV/HIV group, $p=0.89$) and not significantly different between the two groups (3.36 kPa and 1.54 kPa, for the HCV and the HCV/HIV group, respectively) (Table 3). The fibrotic stage did not change in most patients in both groups (68.8% and 78.4%, respectively) (Table 4). Only 19 patients exhibited an increase in the fibrosis stage, mostly 1 stage (Table 5). There were neither differences in baseline demographic data nor an increase in the fibrosis stage between both group (data not shown).

Table 2. Liver stiffness at baseline and at 6-month interval.

Liver stiffness	HCV mono-infection		HCV/HIV co-infection	
	Initial	6th month	Initial	6th month
All (kPa)	17.11 (13.49)	20.03 (17.29)	9.04 (9.07)	10.46 (12.59)
F1 (n, %)	16 (22.9)	16 (23.9)	36 (65.5)	31 (59.6)
F2 (n , %)	13 (18.6)	7 (10.4)	9 (16.4)	10 (19.2)
F3 (n, %)	7 (10.0)	7 (10.4)	1 (1.8)	2 (3.8)
F4 (n , %)	34 (48.6)	37 (55.2)	9 (16.4)	9 (17.3)
Total, (n)	70	67	55	52

Table 3. Liver stiffness changes.

Baseline fibrosis stage	HCV mono-infection	HCV/HIV co-infection	*p*-value
All (mean, SD)	3.46 (7.86)	1.54 (5.55)	0.107
All who increased	5.95 (7.98)	3.49 (5.88)	0.895
All who decreased	-2.56 (2.95)	-2.18 (2.13)	0.044
F1	1.48 (2.25)	0.95 (1.82)	
F2	0.05 (3.41)	-1.36 (1.70)	
F3	3.56 (3.56)	0.6 (0.0)	
F4	5.79 (10.44)	7.74 (12.29)	

*mean (SD), Mann-Whitney

Table 4. Fibrosis stage changes.

Fibrosis stage change	HCV mono-infection (n, %)	HCV/HIV co-infeciton (n, %)	*p*-value
-1	8 (12.5)	4 (7.8)	0.83
0	44 (68.8)	40 (78.4)	
1	8 (12.5)	5 (9.8)	
2	3 (4.7)	1 (2)	
3	1 (1.6)	1 (2)	

Table 5. Fibrosis progression according to the initial fibrosis stage*.

Initial stage	+1 stage		+2 stages		+3 stages		Total
	HCV	HCV/HIV	HCV	HCV/HIV	HCV	HCV/HIV	
F1	3 (37.5)	5 (100)	1 (33.3)	1 (100)	1 (100)	1 (100)	12
F2	1 (12.5)	0	2 (66.7)	0	0	0	3
F3	4 (50)	0	0	0	0	0	4
F4	0	0	0	0	0	0	0
Total	8	5	3	1	1	1	19

*number (%)

Table 6. Death cases.

Sex/age	group	HCV viral load	CD4 count, %	Last HIV viral load	TE value	Cause of death
F/69	HCV	<600	NA	NA	28.0	*E.coli* septicemia
M/63	HCV	pending	NA	NA	37.4	EV bleeding
M/50	HCV/HIV	pending	57, 2%	61	4.2	HAP
M/52	HCV/HIV	pending	375, 21%	23	23.4	HAP

There were also no differences in the change of LS and fibrosis stage between the two groups, when comparing subject with the same baseline fibrosis stage and the same duration of HCV infection (data not shown).

The success rate of fibroscan was 95% (125/131) and 98% (119/121) for the first and the second LSM. In the 6 unsuccessful LSM, the results were invalid in 3 patients (50%), fatty skin in 2 patients (33%) and small intercostal space in 1 patient (12%).

Mortality

There were 4 deaths, 2 in each group during the 6-month follow-up period. First, female 69 years old, her LS was 28.0 kPa. The cause of death was E.coli septicemia without clinical-detected ascites and no definite source of infection. She had history of prior EV bleeding. Second, male 63 years old, his LS was 37.4 kPa and hospitalized due to massive EV bleeding and then developed multiorgan failure. Third, male 50 years old with HCV/HIV co-infection, his LS was 4.2 kPa. He was not well-control HIV infection with viral load 61 copies/mL and CD_4 count was 57 (2%) after 10 year of HAART. He was hospitalized because multiple cranial nerve palsy and developed severe hospital acquired pneumonia (HAP) and death. The last one was 52 years old male with HCV/HIV co-infection, CD_4 count was 375 (21%) and last HIV viral load was 23 copies/mL. He developed progressive mechanical dysphagia. The diagnosis was esophageal papilloma by EGD finding and pathological report. After prolong hospitalization for improve him the nutrition, he also developed HAP and death (Table 6).

DISCUSSION

There had been little data on monitoring liver fibrosis progression using the new noninvasive çtransient elastographyé method, especially in untreated HCV patients. Our prospective cohort study was designed to address this issue.

At baseline, the HCV disease activity was apparently more severe in the HCV mono-infection group. This was reflected by a greater number of patients with high viral load, longer duration of infection, worse liver synthetic function and higher LS (mostly of them were F4). Both groups increased LS were noted in both groups, but changes after 6 months were comparable.

There was only modest change of LS after 6 months in both groups, without statistical difference, irrespective of baseline fibrosis stage or duration of HCV infection. Our finding was in contrast with previous study showing a more rapid progression in HCV/HIV co-infection groups. In our HCV/HIV co-infection groups, however, the HIV infection in most subjects was well-controlled, with undetectable HCV RNA in 60% of cases. This could explain why fibrosis progression was not more rapid than in the HCV mono-infection group.

Macias et al[5]. found that fibrosis progression by at least 1 stage was 44% in HCV/HIV co-infection. In this study, 3-year interval paired liver biopsy was obtained[5]. In other liver-biopsy studies with 3 to 4 year interval times, about 25-50% significant fibrosis progression was reported[6,9]. Interestingly, 12.5% (7/56) of patients in our HCV/HIV group showed at least 1 fibrosis stage progression within 6 months only, although without significant difference from the HCV mono-infection group. Notably also, 67.8% (78/115) of patients showed increased LS, and 19 patients showed fibrosis progression, more than half of whom (11/19) progressed to advanced fibrosis, as diagnosed with high accuracy by transient elastography method[7]. It is possible that LS in such patients may progressively increase leading to clinical consequence.

Two patients died after esophageal variceal bleeding episode. The LS were >21 kPa, in keeping with previous report, prediction of esophageal varices re-

quiring therapy (sensitivity and NPV 100%)[10]. The cause of death in these 2 cases was çliver-related mortalityé. Two other dead patients with low LS died from non liver-related causes.

There were limitations of our study. First, the route of infection and the duration of infection were estimated from the time to risk of HCV acquisition event and could be a recall bias. Second, in some patients, there might be more than one route of HCV acquisition, and the longest duration was used to calculate the duration of infection. Third, we had no liver biopsy histology to compare with LSM result. However, many previous studies had shown high diagnostic accuracy of LSM, and the aim of our study was to study fibrosis progression from baseline measurement. Study patients fasted at least 4 hours before the test, and changes of AST and ALT values during the 6-month interval were comparable in both groups. We consider our study results reliable.

In conclusion, in our study a 6-month interval fibrosis progression was demonstrated by using LSM, although no difference were noted between the two groups. A study with longer follow-up is needed.

REFERENCES

1. Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. Liver Transpl 2003;9:331-8.
2. Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. Hepatology 2008;48:418-31.
3. Rosen HR. Clinical practice. Chronic hepatitis C infection. N Engl J Med 2011;364:2429-38.
4. Koziel MJ, Peters MG. Viral hepatitis in HIV infection. N Engl J Med 2007;356:1445-54.
5. Macias J, Berenguer J, Japon MA, Giron JA, Rivero A, Lopez-Cortes LF, *et al*. Fast fibrosis progression between repeated liver biopsies in patients coinfected with human immunodeficiency virus/hepatitis C virus. Hepatology 2009;50:1056-63.
6. Schiavini M, Angeli E, Mainini A, Zerbi P, Duca PG, Gubertini G, *et al*. Risk factors for fibrosis progression in HIV/HCV coinfected patients from a retrospective analysis of liver biopsies in 1985-2002. HIV Med 2006;7:331-7.
7. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology 2012;142:1293-302 e4.
8. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, *et al*. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343-50.
9. Sulkowski MS, Mehta SH, Torbenson MS, Higgins Y, Brinkley SC, de Oca RM, *et al*. Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. AIDS 2007;21:2209-16.
10. Pineda JA, Recio E, Camacho A, Macias J, Almodovar C, Gonzalez-Serrano M, *et al*. Liver stiffness as a predictor of esophageal varices requiring therapy in HIV/hepatitis C virus-coinfected patients with cirrhosis. J Acquir Immune Defic Syndr 2009;51:445-9.