ORIGINAL ARTICLE

Serum biomarkers and transient elastography versus histopathology for assessment of post living donor liver transplantation (LDLT) Hepatitis C virus recurrence

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ABSTRACT

This study aimed to detect fibrosis in Hepatitis C virus (HCV) infected liver transplant recipients using serum markers and Fibroscan, and to validate the diagnostic accuracy of these non-invasive methods in comparison with liver biopsy.

Patients and methods: Twenty-five consecutive patients with Hepatitis C virus related end-stage liver disease who underwent living donor liver transplantation (LDLT) were scheduled for assessment of hepatic fibrosis through assaying serum fibromarkers, fibroscan and protocol liver biopsy after liver transplantation to examine Hepatitis C virus recurrence and to assess stage of fibrosis and histological activity index using Metavir score.

Results: It was found that Fibrosis-4 index (FIB-4) showed good concordance to liver biopsy examination as regards F1. On the other hand, there was no concordance of FIB-4 to other grades of liver fibrosis. Age to Platelet Ratio Index (APRI) showed excellent concordance to liver biopsy examination as regards different grades of liver fibrosis. Excellent concordance to liver biopsy examination was found considering serum values of Hyaluronic Acid (HA). Liver stiffness measurement by Fibroscan agreed with pathological examination of liver biopsies results in 44% of cases.

Conclusion: Non-invasive techniques as serum biomarkers and fibroscan may predict recurrence of hepatitis and liver fibrosis among chronic Hepatitis C virus patients who underwent living donor liver transplantation. Non-invasive markers including APRI score and HA can predict liver fibrosis precisely.

Key words: HCV, Living donor liver transplantation (LDLT), Serum markers, Fibroscan, Liver fibrosis.

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INTRODUCTION

Recurrence of hepatitis in most Hepatitis C virus (HCV) transplanted livers is progressive with development of bridging fibrosis and cirrhosis in 20–54% at 5 years and 32–51% at 7 years (1). Acceleration of fibrogenesis after liver transplantation may be due to: (i) poor relation between liver function tests and pathological findings of liver biopsies, both in HCV and non-HCV recipients (2, 3). In a previous study, 165 liver biopsies were investigated at the time of normal liver function tests, pathological abnormalities were detected in almost one third of biopsies. The pathological findings involved liver steatosis, low-grade/low-stage recurrent hepatitis C or primary biliary cirrhosis, or central venulitis (4); (ii) the acceleration of fibrogenesis in transplant compared to non-transplant patients, with medium annual rates ranging from 0.2 to 0.8 Metavir stages/year (5) compared to 0.1–0.2 in non-transplanted, immune competent patients (6). Definitely, the liver fibrogenesis that seems to be detected early after transplantation (7, 8), within the first year has been found to be tightly related to development of cirrhosis with great impact on graft rejection and patient survival. Moreover, some factors could be used to predict recurrence of hepatitis after liver transplantation. These factors are: the degree of necroinflammation in early biopsis, the donor age, viral load, the degree of immunosuppression and surgical complications occurring during the first months' post-transplantation, mainly biliary complications (9, 10).

This study aimed to detect fibrosis in HCV infected liver transplant recipients with serum markers and Fibroscan and to validate the diagnostic accuracy of these non-invasive methods in comparison with liver biopsy.

PATIENTS AND METHODS

Ethical approval was obtained from the Medical Ethical Committee, National Research Center (study approval number: 11128). Informed written consent was obtained from all the study's participants (11). Twenty five consecutive patients with HCV related end-stage liver disease who underwent living donor liver transplantation (LDLT) were scheduled for assessment of hepatic fibrosis through measuring serum fibromarkers and protocol liver biopsy 6 months after liver transplantation to examine for HCV recurrence and to assess for stage of fibrosis and histological activity index using Metavir score, and at the same time to exclude other causes of liver graft dysfunction as rejection or drug toxicity. Patients with the following circumstances were excluded from the study: Liver recipients < 18 years old, Survival for less than 3 months post LT, HBV coinfected with HCV, any other aetiology for pretransplant liver disease other than HCV, Rejection, Body Mass Index (BMI) > 35, contraindications for liver biopsy (i.e., bleeding tendency or biliary dilatation) and presence of ascites at time of performing fibroscan.

The following basic laboratory tests were done for all participants after liver transplantation: Fasting blood glucose, aspartate amino transferase (AST), serum alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin (Total & Direct), total protein, albumin, complete blood count (CBC), prothrombin time (PT), INR, urea, creatinine, uric acid, sodium, potassium, calcium, tacrilimus or cyclosporine level and viral load.

Assessment of hepatic fibrosis

Simple scores for fibrosis: aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio and AST to platelet ratio index (APRI) was calculated as follows: (AST/upper limit of normal)/platelet count (expressed as platelets × 109/L) × 100, modified APRI calculated as follows: [Age (y) x (AST/upper limit of normal] / [Serum albumin (g/dl) x platelet count (expressed as platelets × 109/L) × 100](12), FIB-4 calculated as follows: [Age(y) x AST(U/L] / [platelet count (expressed as platelets × 109/L X square root of ALT(U/L)]. α-2-macroglobulin, haptoglobin, apolipoprotein A1 and hyaluronic acid levels were detected by Enzyme-Linked Immunosorbent Assay (ELISA) provided by Assay Max (Assaypro, Mo, USA) (13).

Transient Elastography (TE) or Fibroscan

Fibroscan® (Echosens Co., Paris, France) evaluated the elasticity of the liver by measuring the velocity of a low-frequency shear wave crossing the hepatic parenchyma.

Conversion of fibroscan results

Stiffness (kPa) into stages of fibrosis (Metavir), 0-5.4: F0 (absent fibrosis), 5-5.9: F0-F1(absent to mild fibrosis), 6-6.9: F1 (mild fibrosis), 7-8.7: F1-F2 (Mild to significant fibrosis), 8.8-9.4: F2 (significant fibrosis), 9.5-12.4: F3 (marked fibrosis), 12.5-14.4: F3-F4 (marked fibrosis to cirrhosis) and more than 14.4: F4 (cirrhosis) (14).

Liver biopsy examination

Liver biopsies were performed under local anaesthetic and 5mg diazepam or midazolam was given and patient was fasting overnight before the procedure. Biopsies were taken with Tru-cut needle (16 or 18 gauge) and under ultrasonographic guidance. Liver specimens were kept in formalin. Samples were processed at the pathology department and stained with haematoxylin & eosin and Masson's trichrome. A pathologist who did not know results of transient elastography values examined all histological samples. Necro-inflammatory activity and fibrosis stage were scored using the Metavir score that used two standardized numbers: ″grade″ to indicate degree of inflammation (tissue swelling and irritation) and "stage" indicating the degree of fibrosis (tissue scarring due to prolonged inflammation) (15).

Statistical Methods

SPSS version 14 was used (statistical package for the social sciences). Patients' categorical variables were presented by number and percent. They were analysed using Chi-square or Fischer's exact test when appropriate. Quantitative variables were presented by mean and standard deviation (SD). They were correlated with fibrosis stages using Spearman correlation and with stiffness levels in fibroscan using Pearson correlation. In all tests, p values were considered significant if less than 0.05.

RESULTS

Table 1. showed 88% of recipients were males and 12% were females, their mean age was 51.24±4.47 years, while the mean age of donors was 32.08±3.55 years. The mean survival of recipients was 31.08±17.06 months, the mean Child score of of the recipients was 9.88±,1.33 while the mean Meld score was 17.24±3.32. 69% of recipients were non-diabetics, while 32% were diabetics. 52% of recipients had no post-operative biliary complications, while 48% had biliary complications. Metavir score in liver biopsy stage of fibrosis was F1 in 92% of recipients, F2 in 4% of cases, and F4 in 4% of cases. The mean of timing of liver biopsy post-transplant was 16.8 months as shown in Table (2). Serum biomarkers levels were illustrated in Table.3. Mean values of Liver stiffness (KPa) in studied patients were 8.76 KPa, 5/25 (20%) patients showed no fibrosis (F0) while 11/25 (44%) showed mild-moderate fibrosis (F1-2) and 9/25 (36%) showed severe fibrosis (F3-4) as shown in Table 4.

It was very important to address the validity of non-invasive techniques in diagnosis of liver fibrosis. FIB-4 showed good concordance to liver biopsy examination as regards to F1. On the other hand, there was no concordance of FIB-4 to other grades of liver fibrosis. APRI Showed excellent concordance to liver biopsy examination as regards different grades of liver fibrosis. Excellent concordance to liver biopsy examination was found considering serum values of HA as shown in Table 5. Liver stiffness measurement by Fibroscan agreed with pathological examination of liver biopsies results in 44% of cases and disagreed in 56% of cases as shown in Table 6. Considering Metavir score as a reference landmark of the current study, there was a significant inverse correlation between Age/PLT Index and grades of liver fibrosis. There was a significant direct correlation between grades of liver fibrosis and AST/ALT ratio, APRI, Modified APRI, FIB-4 and Liver Stiffness by Fibro scan. Other markers did not show any significant correlation to grades of liver fibrosis as shown in Table 6.

Table 1. Patient Characteristics

Variables (mean ± SD)	Patients (N=25)	Reference Range
Age (years)	51.24 ± 4.47	-
Gender: Male n= (%) Female n= (%)	22 (88) 3 (12)	-
Hb (mmol/L)	8.29 ± 1.22	8.69 - 10.86
WBC (cells*10^9/L)	5.46*10^9±1.96*10^9/L	4.5*10^9/L-11*10^9/L
PLT (count/L)	157.08 ± 63.22	150000 - 450000
PC %	88.46 ± 12.18	75 - 100
INR	1.10 ± 0.12	0.8 - 1.1
T. Bilirubin (µmol/L)	16.93 ± 9.06	3.42 -2 0.52
D. Bilirubin (µmol/L)	6.84 ± 4.96	< 5.13
ALT (µkat/L)	0.97 ± 0.73	0.12 - 0.91
AST (µkat/L)	0.87 ± 0.51	0.13 - 0.8
Alk. P (µkat/L)	3.43 ± 2.03	0.73 - 2.45
T. Ptn (g/L)	73.0 ± 7.3	60 - 83
ALB (g/L)	40.8 ± 6	35 - 55
Urea (mmol/L)	13.36 ± 6.83	2.14 - 8.57
Creatinine (µmol/L)	84.0 ± 30.95	53.05 - 97.26
Uric acid (µmol/L)	362.83 ± 108.85	208.18 - 434.2
Viral Load (copies/mL)	282371 ± 330042	-
Na (mmol/L)	140.88 ± 5.02	135 - 145
K (mmol/L	4.60 ± 0.54	3.6 - 5.2
Ca (mmol/L)	8.61 ± 0.55	8.5 - 10.2
Biliary complications: Yes NO(%) No NO(%)	 12(48.0) 13(52.0)	-

Hb: haemoglobin, WBC: white blood cells, PLT: platelets, PC: prothrombin concentration, INR: international randomized ratio, T. Bilirubin: total bilirubin, D. Bilirubin: direct bilirubin, ALT: alanine transaminase, AST: aspartate transaminase, Alk. P: alkaline phosphatase, T.Ptn: total protein, Alb: albumin.

Table 2. Stages of fibrosis evaluated by Liver biopsy in studied patients

		Frequency	Percent	
Stage	F1	23	92.0	
	F2	1	4.0	
	F4	1	4.0	
	Total	25	100.0	
Timing of biopsy post-transplant (months)	Mean	SD	Minimum	Maximum
	16.80	13.98	3.00	65.00

Table 3. Values of Non-invasive indices in studied patients

Variables	Mean	SD	Minimum	Maximum	Reference Range
Age/PLT Index	5.71	1.73	2.00	8.00	-
APRI	0.39	0.30	0.14	1.49	-
Modified APRI	5.52	5.92	1.47	28.77	-
FIB-4	2.78	2.22	0.97	11.75	-
α-2-macroglobulin (g/L)	3.07	4.01	1.19	22.01	1.12 -3 .54
Haptoglobin (g/L)	4.54	5.29	0.11	25.88	0.41 - 0.17
Apolipoprotein A1(g/L)	12.98	5.15	1.26	21.43	0.75 - 1.75
Hyaluronic Acid (g/L)	0.00004947	0.00003208	0.00002563	0.00018188	0.0000051 - 0.000062

Age/PLT Index: age to platelets index, APRI: AST to platelet ratio index

Table 4. Stages of fibrosis among studied patients measured by Fibroscan

	Stages	Frequency	Percent	
Valid	F0	5	20.0	
	F0 - F1	1	4.0	
	F1	5	20.0	
	F1 - 2	1	4.0	
	F2	3	12.0	
	F2 - F3	1	4.0	
	F3	3	12.0	
	F3 - F4	4	16.0	
	F4	2	8.0	
	Total	25	100.0	
Fibroscan Stiffness (kPa)	**Mean**	**SD**	**Minimum**	**Maximum**
	8.76	4.00	4.10	17.50

Table 5. Concordance of FIB-4 score, APRI & Hyaluronic acid with liver biopsy results

		Liver Biopsy			
		F1	F2	F4	Total
FIB-4	<1.45 (≤F1)	4	1	0	5
	1.45-3.25(≥F2)	13	0	0	13
	>3.25 (F3-4)	6	0	1	7
APRI	<0.7 (≤F1)	22	1	0	23
	0.7-1 (≥F2)	1	0	0	1
	>1 (F3-4)	0	0	1	1
Hyaluronic Acid	<113 (<F1)	22	1	1	24
	113-181 (>F1)	1	0	0	1
Total number of cases					25

Table 6. Agreement between liver stiffness measured by fibroscan and liver biopsy

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Agreement	11	44.0	44.0	44.0
	Disagreement	14	56.0	56.0	100.0
	Total	25	100.0	100.0	

Table 7. Correlation between stages of fibrosis by Metavir Score and non-invasive indices of fibrosis

	Correlation coefficient (r)	**P value**
Age/PLT Index	-0.56*	0.025
AST/ALT ratio	0.590**	0.002
APRI	0.681**	0.0001
Modified APRI	0.740**	0.0001
FIB-4	0.764**	0.0001
Liver Stiffness (kPa)	0.429*	0.032
α-2-macroglobulin (g/L)	0.001	0.999
Haptoglobin (g/L)	-0.127	0.546
Apolipoprotein A1 (g/L)	-0.076	0.717
GGT (µkat/L)	-0.050	0.813
Hyaluronic Acid (g/L)	-0.135	0.521

AST/ALT ratio: Aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio, APRI: AST to platelet ratio index, GGT: gamma glutamyl transaminase. *: p value ≤0,05 is significant, **: p value ≤0.001 is highly significant. The SI units of the markers of serum that were measured by ELISA (g/L)

DISCUSSION

In our series, none of the recipients who developed clinical HCV recurrence developed allograft cirrhosis over mean follow up period of 16.8 months and 92% of our patients developed only mild fibrosis (F1). In previous study done by our team published in 2009 (16) HCV recurrence was shown in 31% of 74 patients followed up for 36 months and 91% showed stage of fibrosis <F2. Despite the great concern in literature about severity of HCV recurrence post liver transplantation due to accelerated fibrosis progression, in our cohort we did not have severe fibrosis over follow up period.

However, in our series some variables showed the same findings of inaccuracy while others could precisely predict fibrosis stages. One of these indices (FIB-4), which depend on age, AST, ALT & platelets count predicted only 4 patients with Fib ≤ F1 while it overestimated fibrosis stages in 6 patients, only 1 of them was shown to be F4 by liver biopsy, the approximate predictability of FIB-4 in comparison with liver biopsy was only 6/25 (24%). Previous studies reported that APRI score, FORNS index and FIB-4 score were less accurate in determining early grades of fibrosis than cirrhosis (17, 18). On the other hand, Knop and his co-workers concluded that only FIB-4 score showed AUROC values >90% to predict cirrhosis, even better at excluding than diagnosing cirrhosis (NPV and PPV 94.7% and 75.1%) using a cut-off value >3.25 (19).

Garcia-Tsao and his co-workers explained the differences in the prognostic value of FIB-4 when compared to fibrosis staging by liver biopsy by the different ability of both techniques to interpret changes in portal hypertension, the most evident predictor of outcome in compensated liver disease (20). It was known that most of the histological staging systems for fibrosis, such as Metavir, involve all types of cirrhosis into a single grade. Such an approach significantly decreases inter-observer differences between histopathologists but does not consider the variant clinical stages of cirrhosis (21). However, FIB-4 is determined with biochemical variables that may be related to changes associated with liver function deterioration, such as platelet count, which is considered as a marker of advanced liver fibrosis (22) Moreover, serum values of liver enzymes as aspartate aminotransferase and alanine aminotransferase correlate with stages of liver inflammation (23).

Results of APRI score were in agreement with liver biopsy results in almost all of cases and it was able to predict fibrosis stages precisely. As regards Age/PLT Index and AST/ALT ratio they showed rough correlation with fibrosis stages assessed by Metavir score with no adequate results to conclude its ability to precisely predict each stage. In the study of Wai et al., they stated that APRI value can be used to predict fibrosis and cirrhosis (24,25) However, in our study, there was no statistically significant correlation between APRI score and the degree of fibrosis. Previous studies showed that serum biomarkers such as PIIINP and various types of collagens, HA has a more significant accuracy to diagnose cirrhosis (26). Guha and his co-workers suggested that serum biomarkers (Hyaluronic Acid, Apolipoprotein A1, α-2-macroglobulin, Haptoglobin, Gama Glutamyl Transferase (GGT) could be used as diagnostic especially when included in Algorithms as Fibrotest. However, high scuttle of these biomarkers three months after liver transplantation may prevent accurate differentiation between cases with mild or advanced recurrence of hepatitis at this time point (27). On the other hand, Martinez and his co-workers found that serum values of these biomarkers at 6 and 12 months distinguished between cases with mild and severe liver fibrosis. They proved that 6 months is the best time point to diagnose progressive hepatitis C recurrence (28). In the current study, it was found that serum values of Hyaluronic Acid could diagnose grades of liver fibrosis as pathological examination did. Our findings were supported by Attallah and his co-workers

who concluded that HA was the most accurate biomarker among HA, N-acetyl-β-D-glucosaminidase, glucuronic acid, glucosamine and AST/ALT ratio (29) The validity of serum HA levels to assess liver necroinflammatory injuries, has also been confirmed in other studies (30).

In contrast to Biochemical Indices, Transient elastography is not affected by clinicoserological variables and directly assesses liver stiffness: this justifies the higher accuracy in comparison to clinicoserological variables in the liver transplantation setting (31). Rigamonti and his co-workers proved that Transient elastography has an efficient ability in ruling out significant fibrosis rather than ruling it in, as detected by negative predictive values higher than positive ones at the different thresholds (32). Existing comprehensive intervention in liver stiffness range of cases classified as F2–F3, making transient elastography more susceptible to confirm presence or absence of evident fibrosis rather than exactly discriminating patients to individual liver fibrosis stages (33). In our series Transient elastography (TE) showed statistical agreement with liver biopsy in 44% of our patients, however as said before an extensive overlap exists in liver stiffness ranges of patients classified as F1-2 & F3-4, considering this overlap it could predict fibrosis stages F0-1 and F1-2 in 15/25 (60%) of our patients, it over estimates fibrosis stages in 8 cases (F3/F3-4/ F4 in 3/4/1), however if we consider TE for treatment decision it could predict significant fibrosis that give rise to the urgency and importance of considering antiviral therapy. In an era of combined interferon therapy and before discovery of Direct Acting Antiviral Drugs, decision of starting therapy for HCV recurrence post liver transplantation was depending on whether this patient developed significant fibrosis (> F1) or not to avoid exposing patient to hazard and risk of severe interferon related adverse effects. In an era of DAA, antiviral therapy is considered 6 months post transplantation regardless fibrosis stages due to relative safety and efficacy of DAA, however detection of stage of fibrosis would help to guide follow up plan for example if patient had severe fibrosis (F3/4) close monitoring will be required to for early detection of complications despite adequate viral eradication; namely signs of portal hypertension or HCC. For this non-invasive prediction of fibrosis will be important to avoid unnecessary liver biopsy unless other diagnoses for liver dysfunction namely rejection or biliary complications or drug toxicity which could not be diagnosed except by liver biopsy. However, it is considered that relatively small sample size and a disproportionate gender distribution may be a limitation of the current study.

CONCLUSION

Non-invasive techniques as serum biomarkers and Fibroscan may predict recurrence of hepatitis and liver fibrosis among chronic HCV patients who underwent living donor liver transplantation. APRI score and HA can predict liver fibrosis precisely.

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