Viral Load and Genotype Matter in Hepatitis C Virus Related Heart Disease in Cirrhotic Patients

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Abstract: Background: Although the term cirrhotic cardiomyopathy expresses cardiac changes associating liver cirrhosis unrelated to its aetiology, hepatitis C virus HCV was suggested to play a role in cardiomyopathy and/or myocarditis. However the existence of such direct viral effect on the heart in HCV induced liver cirrhosis LC patients remains to be verified**. Rationale of the current study** was to figure out the influence of the viral load, and genotype in addition to Child Pugh CP score on cardiac structural and functional changes in HCV induced LC patients. Sixty patients were classified according to viral genotype into 4a and non-4a groups. Viral load and the degree of liver impairment according to CP score were also verified in these patients. Data were correlated with echocardiographically assessed left and right sided chambers' structures and functions**. Main Results:** E/A ratio as an indicator of left ventricular diastolic function showed negative correlation with the viral load and CP score. Ejection fraction of HCV induced LC patients correlated positively with viral load and CP score. Patients who belonged to non-4a genotype group showed significantly larger left ventricular end-diastolic dimensions and left atrial dimensions compared to the 4a group. Right ventricular dimensions correlated positively with CP score. **Conclusion:** The current study showed that both viral load and genotype had an add-on effect on the expected cirrhotic cardiomyopathic changes in HCV induced LC patients.
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1. Introduction

A hyperdynamic cardiovascular state was noted in liver cirrhosis patients more than fifty years ago (1). It was attributed to decreased systemic vascular resistance (2) as well as a shift of blood from systemic to splanchnic circulation (3). Later on a patho-physiological entity termed cirrhotic cardiomyopathy was described in cirrhotic patients as diastolic dysfunction, associated with increased cardiac output under resting conditions, but subnormal response to stress (4). It was initially misdiagnosed as alcohol induced heart disease in alcoholic cirrhosis patients (2). Later on it was found to include functional, structural and electrophysiologic cardiac changes regardless of the aetiology of liver cirrhosis (5). Cellular changes of cirrhotic cardiomyopathy were attributed to sympathetic overstimulation with associated beta receptor downregulation and postreceptor dysfunction (5). Cardiodepressent effect (1) of endotoxins, endothelins, cytokines, bile acids, and humoral factors as nitric oxide, carbon monoxide, and endogenous cannabinoids were also determinant factors in cellular injury. Hepatitis C virus HCV was reported in previous studies to induce dilated cardiomyopathy (6, 7), hypertrophic cardiomyopathy (7- 9), right ventricular arrhythmogenic cardiomyopathy (10), myocarditis (7, 11), and cardiac fibrosis (12, 13). Being a major cause of liver cirrhosis (14) HCV might have an add-on effect on cardiac structural and functional changes associating HCV induced liver cirrhosis LC.

There are at least six known genotypes of HCV, with more than 50 subtypes all over the world (15). Egypt has the highest HCV infection prevalence (16) with special predilection of 4a viral genotype (17, 18).

The objective of the current study was to determine any peculiar effect of HCV viral load and/ or genotype on cardiac structural and functional changes in HCV induced liver cirrhosis patients, superimposed on suspected cirrhotic cardiomyopathy, as assessed by conventional Transthoracic Two-Dimensional 2D Echocardiography.

2-Subjects and Methods:

2-i. Study Population:

In a case control hospital based observational study 60 HCV induced liver cirrhosis LC Egyptian patients were recruited from Internal Medicine Department outpatient clinic of Kasr El-Aini tertiary care University Hospital from December 2010 to December 2011. Twenty age-matched healthy anti-HCV antibody and Hepatitis B virus HBV surface

antigen seronegative medical personnel participated as control group. LC diagnosis was based on established clinical, biochemical, and ultrasonography criteria, but liver biopsy was not done. Patients were subjected to thorough clinical evaluation, chest X-ray, and electrocardiography. Body mass index was calculated as weight in kilograms divided by square the height in meters (kg/m^2). Blood sampling for hemoglobin, prothrombin time, serum sodium, potassium, urea, creatinine, albumin, bilirubin as well as fasting and 2 hours postprandial blood glucose estimation and abdominal ultrasound imaging were performed.

HCV induced LC patients were categorized according to modified Child Pugh CP classification into A, B, and C, depending on serum bilirubin, serum albumin, prothrombin time, ascites, and hepatic encephalopathy. According to point score 5-6 points were considered CP grade A ,7-9 points CP grade B, and 10-15 points CP grade C (19).

Patients were subjected to quantitative assessment of HCV viraemia level by real time polymerase chain reaction RT-PCR to detect the viral load, and viral gene sequencing to determine the viral genotype. All patients were naive to HCV anti-viral medications. Transthoracic two-dimensional 2D echocardiography was performed for patients and controls.

2-ii. Exclusion Criteria:

Excluded from the current study were those with known pulmonary or heart disease whether valvular, ischaemic, rhythm abnormalities, or hypertensive, with blood pressure cutoff level of < 120/80 as previously set by JNC VII (20), diabetic people according to ADA diagnostic criteria (21), those with renal impairment, alcohol consumers, and current or ex-smokers over the last 10 years. Those with concomitant hepatitis B infection and those with a poor acoustic cardiac window were also exempted.

Informed written consents were obtained from the participants. The study protocol agreed with Helsinki declaration, and was approved by the scientific board of Internal Medicine department and Committee of Research Ethics; Faculty of Medicine – Cairo University – Egypt.

2-iii. HBV surface Antigen

HBV surface Antigen was determined by reverse passive haemagglutination assay (Murex Diagnostics Limited, Dartford, UK) according to manufacturer's instructions.

2-iv. Anti-HCV antibodies

Anti-HCV antibodies were assessed using a commercial ELISA test from Abbott-Murex (Murex anti-HCV version 4.0).

2-v. Quantitative measurement of HCV viral load:

Viral load estimation was carried out by Taqman Real Time Polymerase Chain Reaction RT-PCR system using method of Martell *et al.* (22). Viral load was quantified using the light cycler Taqman mater mix kit (Roche diagnostic GmbH, Mannheim, Germany) on Roche light cycler version 2.0. Each specimen was analyzed in duplicate and the mean value reported as the viraemic level in the serum. The unit of the HCV RNA quantification was IU/ml. RT-PCR was performed in the 5' untranslated region (5'UTR) as per manufacturer's instructions (95 ºC for 20 seconds, followed by a further 40 cycles at 95 ºC for 10 seconds, 58 ºC for 15 seconds, and 10 seconds at 72 ºC).

2-vi. HCV Viral Genotype Determination:

Genotypes were assessed by second-generation Line Probe Assay INNO-LiPA HCV 2.0 (Innogenetics Belgium), based on reverse-hybridization of PCR products using oligonucleotide probes in the 5′NCR and the core-coding region. The amplified HCV DNA mixtures were added to denaturation solution for 5 minutes and then hybridization solution were added to each test trough. INNO-LIPA HCVII strips were numbered and completely submerged into the solution in each test trough. Each strip has 3 control lines and 22 parallel DNA lines containing sequences specific for HCV genotypes 1 to 6. Test troughs tray was placed in shaking water bath to allow hybridization. After hybridization the strips were adequately washed using rinse solution, then conjugate was added and strips were incubated for 30 min in an incubator shaker. Pattern of the purple brown lines on the strips were compared with interpretation table to determine HCV genotype.

2-vii. Two Dimensional Echocardiography:

Transthoracic two dimensional 2D, M-mode, and Doppler Echocardiography were performed by one experienced physician who was blinded to the participant condition at the time of the study, using Vivid 3N (General Electric) machine equipped with 2.5 MHz transducer. Parasternal long- and short-axis views as well as apical 4- and 2-chamber views were obtained. Cardiac chambers dimensions were measured according to the American Society of Echocardiography criteria (23) using M-mode method (24). Left ventricular LV systolic and diastolic volumes and ejection fraction were derived from biplane apical (2- and 4-chamber) views with a modified Simpson's rule algorithm (25).

Pulsed-wave Doppler spectral recordings of the transmitral flow were traced at the mitral valve leaflet tips from an apical four chamber view, and used for detection of left ventricular diastolic function (26). Peak E wave for early diastolic filling, peak A wave for late diastolic filling during atrial contraction, E/A

ratio, and deceleration time were recorded. Deceleration time was the interval from the peak E to the decline of velocity to the baseline. When the velocity did not return to baseline, extrapolation of the deceleration signal was performed (27). All measurements were averaged from three cardiac cycles.

2-viii. Statistical Analysis:

Statistical Package of Social Science (SPSS) program version 15.0 was used for analysis of data. Data was summarized as mean ± standard deviation SD for quantitative values, and number and percentage for qualitative variables. Independent sample t-test was used for analysis of 2 quantitative data, one way ANOVA test was used for analysis of more than 2 quantitative data followed by post Hoc Bonferroni test for detection of significance for normally distributed variables. Nonparametric Mann Whitney test and Kruskal-Wallis test were used for quantitative variables which were not normally distributed. Statistical differences between groups were tested using Chi Square test for qualitative variables. Correlations were done to test for linear relations between variables. *P*-value less than or equal to 0.05 was considered statistically significant (28).

3- Results:

The studied HCV induced LC patients were age matched to healthy control subjects. As expected LC patients had lower serum albumin and higher (serum bilirubin, ALT and prothrombin time) compared to healthy control subjects. Patients had higher mean Left atrial dimension LAD and ejection fraction EF but less E/A ratio compared to healthy controls. The rest of the studied parameters did not appear to be significantly different among LC patients compared to healthy control subjects. (Table1)

Nearly 73% of the studied LC patients were infected with HCV 4a genotype while nearly 27% of them were infected with HCV non 4a genotype. Those infected with HCV 4a genotype were older than those infected with HCV non 4a genotype. They had significantly lower viral load, LAD and left ventricle end diastolic dimension (LVEDD). (Table 2)

Among the echocardiographic parameters LAD and EF were positively correlated, while E/A ratio was negatively correlated to the viral load; respectively. (Table 3)

Right ventricular dimension in diastole RVD and EF were positively correlated, while E/A ratio was negatively correlated to CP score; respectively. (Table 4)

Table1. Clinical, Laboratory and Echocardiographic Characteristics of studied subjects

Parameter	HCV induced LC patients	Healthy controls	*p*-value
Number	60	20	
Age(years)	49.4±9.1	47.2±7.60	0.336
Men	33 (55%)	8 (40%)	0.245
Women	27 (45%)	12 (60%)	
BMI(Kg/m^2)	22.93±1.49	22.67±1.17	0.465
HB(g/dl)	12.78±0.58	12.81±0.80	0.892
PT(sec)	15.7±3.38	11.35±1.09	0.000
Bilirubin(mg/dl)	2.58±1.42	0.93±0.26	0.000
Albumin(g/dl)	3.03±0.8	4.41±0.44	0.000
ALT	55.65±44.3	19.55±6.6	0.000
LAD	3.85±0.49	3.31±0.42	0.000
LVEDD	5.08±0.8	4.86±0.52	0.253
LVPWd	0.95±0.09	0.89±0.2	0.966
IVSd	0.95±0.09	0.89±0.15	0.982
RVD	2.07±0.44	2.12±0.47	0.685
EF	71.43±5.0	66.55±6.1	0.001
E/A	1.09±0.42	1.48±0.19	0.000

Data are expressed as mean ±SD, number or percentage as appropriate. BMI: body mass index, HB: hemoglobin, PT: prothrombin time, ALT: alanine aminotransferase, LAD: left atrial dimension, LVEDD: left ventricular end diastolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, IVSd: interventricular septum thickness in diastole, RVD: right ventricular dimension in diastole, EF: ejection fraction, E: peak transmitral flow velocity in early diastole, A: peak transmitral flow velocity in late diastole, E/A ratio, ratio of velocity of E wave to velocity of A wave of Doppler trans-mitral valve flow.

Table 2 .Clinical, Laboratory and Echocardiographic Characteristics of studied HCV positive Liver Cirrhosis patients according to HCV Genotype:

Parameter	HCV 4a genotype	HCV non 4a genotype	*p*-value
Number	44/60(73.33%)	16/60(26.67%)	
Age(years)	51.23±9.07	44.38±7.52	**0.009**
Men	23 (52.3%)	10 (62.5%)	0.481
Women	21(47.7%)	6(37.5%)	
BMI(Kg/m^2)	22.85±1.5	23.15±1.46	0.500
HB(g/dl)	12.78±0.64	12.79±0.47	0.934
PT(sec)	15.73±3.36	15.63±3.54	0.919
Bilirubin(mg/dl)	2.66±1.37	3.19±0.85	0.441
Albumin(g/dl)	2.97±0.78	3.19±0.85	0.358
ALT	59.48±50.15	45.13±18.91	1.000
CP score	8.98±3.04	7.50±2.16	0.101
Viral load(x10^{5}IU/ml)	9.97±8.66	17±13.03	**0.038**
LAD	3.79±0.5	4.03±0.23	**0.015**
LVEDD	4.97±0.83	5.39±0.65	**0.048**
LVPWd	0.93±0.07	0.98±0.12	0.945
IVSd	0.9±0.08	0.96±0.11	0.942
RVD	2.08±0.5	2.03±0.25	0.587
EF	71.55±3.31	71.13±8.22	0.845
E/A	1.09±0.46	1.09±0.29	0.638

Data are expressed as mean ±SD, number, or percentage as appropriate. HCV: hepatitis C virus, BMI: body mass index, HB: hemoglobin, PT: prothrombin time, ALT: alanine aminotransferase, CP score: Child Pugh score, LAD: left atrial dimension, LVEDD: left ventricular end diastolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, IVSd: interventricular septum thickness in diastole, RVD: right ventricular dimension in diastole, EF: ejection fraction, E: peak transmitral flow velocity in early diastole, A: peak transmitral flow velocity after in late diastole, E/A ratio: ratio of velocity of E wave to velocity of A wave of Doppler trans-mitral valve flow.

Table 3. Correlation of Echocardiographic Variables and Viral Load of Liver Cirrhosis Patients:

Parameter	r-value	*p*-value
LAD	**0.264**	**0.042**
LVEDD	0.166	0.206
RVD	- 0.087	0.508
EF	**0.39**	**0.002**
E/A	**- 0.32**	**0.011**

LAD: left atrial dimension, LVEDD: left ventricular end diastolic dimension, LVPWd: left ventricular posterior wall thickness in diastole, IVSd: interventricular septum thickness in diastole, RVD: right ventricle dimension, EF: ejection fraction, E: peak transmitral flow velocity in early diastole, A: peak transmitral flow velocity in late diastole, E/A ratio: ratio of velocity of E wave to velocity of A wave of Doppler trans-mitral flow.

Table 4. Correlation of Echocardiographic Variables and Child-Pugh Score of Liver Cirrhosis Patients

Parameter	r-value	*p*-value
LAD	0.193	0.140
LVEDD	0.255	0.084
RVD	**0.280**	**0.031**
EF	**0.382**	**0.003**
E/A	**- 0.364**	**0.004**

LAD: left atrial dimension, LVEDD: left ventricular end diastolic dimension, RVD: right ventricular dimension in diastole, EF: ejection fraction, E: peak transmitral flow velocity in early diastole, A: peak transmitral flow velocity in late diastole, E/A ratio: ratio of velocity of E wave to velocity of A wave of Doppler trans-mitral flow.

4-Discussion:

In this case control study, cardiac structural and functional changes in HCV induced liver cirrhosis patients, with no history of cardiac disease were explored using conventional transthoracic 2D echocardiography. Relation of these changes to the degree of liver dysfunction, HCV genotype and viral load were specially probed. Both HCV viral load and Child Pugh grade influenced diastolic and systolic left ventricular functions. A significant difference was observed in the effect of various viral genotypes on both LAD and LVEDD with higher dimensions in the non 4a genotype group compared to 4a group.

HCV induced LC patients were found to have lower E/A ratio compared to age matched controls. Lowering of E/A ratio is an early sign of diastolic dysfunction suggested to be the most consistent feature of cirrhotic cardiomyopathy which can be

recorded at rest (3). It is explained by cellular and interstitial oedema (29), which impairs left ventricular relaxation and reduces its compliance. Higher left ventricular end diastolic pressure to end diastolic volume ratio, which means higher impedance to left ventricular filling, is compensated by increase in late diastolic flow velocity (30), resulting into left atrial dilatation (3). This coincided with our finding that LAD was significantly higher in HCV induced LC patients compared to controls. However viral load was significantly negatively correlated to E/A ratio and positively correlated to LAD, respectively. This might be attributed to direct extra-hepatic cardiotropic effect (9, 31) or indirect immunologically mediated role (6) of HCV in the pathogenesis of cellular injury and hence impaired left ventricular diastolic function. Direct HCV myocardial viral replication was also suggested by other investigators (6, 11) as a cause of subclinical myocarditis. In disagreement with previous studies (1) overt concentric left ventricular hypertrophy was not one of our findings, but changes on the cellular level and patchy fibrosis (12, 13) might be sufficient for reduction of left ventricular compliance. Moreover fulfillment of all cirrhotic cardiomyopathy criteria is not a must for its diagnosis (2).

In the present study left ventricular systolic function represented with EF was positively correlated with CP score, which appeared similar to previous studies (2, 32), which reported preserved or even increased systolic function at rest in cirrhotic cardiomyopathy patients. This was explained by reduced afterload due to peripheral vasodilatation in LC patients (33) resulting into the so called hyperdynamic unloaded heart failure state (34). But subtle cirrhotic cardiomyopathic changes are unmasked if a LC patient is challenged by infection (5), exercise (32), trans-jugular intra-hepatic porto-systemic shunting (12, 35), or liver transplantation (36) due to blunted left ventricular response to stress (2), attributed to autonomic dysfunction and impaired volume and baroreceptor reflexes (4). But in the current study 2D Echocardiography was performed at rest, so we were not able to detect this latent systolic dysfunction.

Again in the present study viral load was found to be positively correlated with increased EF in LC patients, a phenomenon that might be explained with contribution of the HCV viraemia level to the hyperdynamic state. In a recent study (37) HCV was shown to be associated with enhanced vascular endothelial growth factor protein expression, and transforming growth factor-beta2 with subsequent activation of endothelial cells and hence vascular proliferation causing hepatic angiogenesis. This effect was found to be peculiar for HCV compared to HBV and autoimmune hepatitis. Although originally the authors addressed it as a mechanism enhancing hepatic neovascularization associating hepatocellular carcinoma, it might also explain aggravation of the hypedynamic state in HCV induced LC patients in a viral dependent manner.

In contradiction to Moller and Henriksen (1) who showed significantly higher LVEDD in their studied LC patients in comparison to controls, we found no significant difference. This might be attributed to discrepancy between both studies in the enrolled population regarding age, gender, ethnicity, LC etiology and HCV genotype. A possible explanation might be that nearly three quarters of our HCV induced LC patients were infected with 4a genotype (i.e. the majority), who had significantly lower LVEDD compared to those infected with non-4a genotypes who represented only one quarter (i.e. the minority). This resulted into reduction of LVEDD difference between patients and controls to insignificant levels.

Subdividing LC patients in the present study according to viral genotype revealed that non- 4a genotype group had significantly higher left sided chambers' dimensions (LAD and LVEDD) compared to the 4a genotype group. Taking age difference into consideration, we expected the reverse as the 4a genotype group showed a significantly higher age, which might have a tendency to higher LAD as detected by previous investigators (38). Therefore other factors as HCV viral load and genotype could explain these findings. HCV viral genotype was mentioned by previous investigators in cardiac diseases as they detected genotype 1b and 2a in the sera of hypertrophic cardiomyopathy patients (9), and genotype 2 in the sera and the myocardium of dilated cardiomyopathy patients (6). The main difference between our study and previous researchers was that they investigated the presence of HCV antibodies or viral genome in patients with established diagnosis of cardiomyopathy. Moreover in the present study non-4a genotype group showed a significantly higher viral load compared to the 4a genotype group.

As regards the right ventricular dimensions in diastole, it showed a significant positive correlation with CP score in the current study. This agreed with previous investigators (39), who explained this by increased nitrate level associating hyperdynamic state in cirrhotic patients. But on the other hand Baik *et al.* (2) stated that the right sided cardiac chambers have normal dimensions and wall thickness in cirrhotic cardiomyopathy patients in absence of porto-pulmonary hypertension.

To the best of our knowledge none of the studies published in English literature investigated

the relation between HCV viral genotype and/or viral load and echocardiographically assessed cardiac structural and functional changes in HCV induced LC patients. The results of this study suggested that HCV genotype probably affected cardiac chambers dimensions rather than LV systolic and diastolic functions. On the other hand viral load appeared to affect left ventricular diastolic function with consequent effect on left atrial dimensions, and enhanced left ventricular systolic function due to expected HCV related aggravation of hyperdynamic state.

Although data of this study might be valuable to the medical literature, yet it has got few limitations. First; the limited number of patients included in this study, which was due to the vast exclusion criteria. Second; the use of conventional Transthoracic 2D Echocardiography instead of Tissue Doppler Echocardiography might have limited our ability to accurately assess diastolic function of subjects included in this study. Third; although cirrhotic patients with history of documented ischaemic heart disease were excluded from this study, a recent study (40) shed light on the fact that end stage liver disease patients have high prevalence of coronary artery disease and that non invasive assessment has limited diagnostic accuracy in these patients. Ischaemic heart disease is a silent disease that can only be accurately excluded invasively, so it might be responsible for some structural and functional changes seen in these patients (40).

5- Conclusion:

The current study showed that HCV viral load and genotype might have unique effects on cardiac structural and functional changes in HCV induced LC patients. This might explain why certain HCV induced LC patients might be more susceptible to overt heart failure when exposed to functional or physical stress than others infected with different HCV genotype or those with non-viral LC, making viral eradication a priority for the former group. Further larger scale comparative studies from different geographical areas which differ in HCV viral genotypes prevalence are recommended.

Authorship:

Abir Zakaria was responsible for performing Echocardiography for participating patients and controls. Ragai Fouda and Mervate Naguib were responsible for clinical, laboratory and ultrasonography data collection and verification. Laila Rashed was responsible for biochemical analysis of collected serum samples. All four authors participated meaningfully in the intellectual content of the study, data analysis, and preparation of the manuscript. All of them certify that they have reviewed the final version of the manuscript, believe it represents a valid study, and approve it for publication.

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Abbreviations:
Hepatitis C virus: HCV
Liver cirrhosis: LC
Child Pugh: CP
HBV: hepatitis B virus
Real Time Polymerase Chain Reaction: RT-PCR
Two-Dimensional: 2-D
JNC: Joint National Committee
ADA: American Diabetes Association
LV: left ventricular
E: peak transmitral flow velocity in early diastole
A: peak transmitral flow velocity after in late diastole
E/A ratio: ratio of velocity of E wave to velocity of A wave of Doppler trans-mitral valve flow
SPSS: Statistical Package of Social Science
ANOVA: analysis of variance
BMI: body mass index
HB: hemoglobin
PT: prothrombin time
ALT: alanine aminotransferase
LAD: left atrial dimension
LVEDD: left ventricular end diastolic dimension
LVPWd: left ventricular posterior wall thickness in diastole
IVSd: interventricular septum thickness in diastole
RVD: right ventricular dimension in diastole
EF: ejection fraction

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