Cardiac Morphofunctional Injuries in Patients with Hepatitis C Virus. Doppler Echocardiographic Evaluation

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Objective: To evaluate possible cardiac morphofunctional injuries in patients chronically infected with the Hepatitis C virus using Doppler echocardiography.

Methods: Control-case observational study analyzing Doppler echocardiographic parameters of 31 patients with chronic hepatitis C, in a non-advanced stage of the disease diagnosed through biopsy (without cirrhosis, hepatocellular carcinoma or hepatic disfunction), and twenty control cases.

Results: There were not statistically significant differences in relation to parietal thickening, cavity diameters, ejection fraction, circumferential shortening, mitral flow velocities and mitral annular systolic and diastolic tissue velocities between both groups studied.

Conclusion: Individuals with hepatitis C on its initial stages did not show cardiac morphofunctional abnormalities in left ventricular Doppler echocardiography evaluation.

Key words: Doppler echocardiography, cardiomyopathy, hepatitis C virus.

Hepatitis caused by C virus (HCV) has high chronification rate: 77% after blood transfusions[1], or 62% after sporadic HCV[2]. It presents mean prevalence from 1.2% to 1.5% in South Europe and Japan, 0.6% in Central Europe and in the United States[3], and is estimated between 1% to 2% in Brazil[4]. Chronified HCV has slow evolution, whereas an average period of ten years elapses from the infection until the manifestation of chronic hepatitis; twenty years for hepatic cirrhosis, and thirty years for the manifestation of hepatocellular carcinoma[5]. Around eleven HCV subtypes are known, six of them in greater depth, and genotype 1 is the most usually found in Brazil. Prevalence studies of positive serology for hepatitis C virus in patients with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) demonstrated significantly elevated rates of its presence[6,7].

Doppler echocardiography is a valuable acknowledged method for the diagnosis of morphological and functional changes of cardiac structures. More recently, tissue Doppler was introduced for myocardial walls or mitral annulus, and through this imaging method diastolic and systolic velocities variables are assessed[8]. In the available medical literature there is not a study including, concomitantly, tissue Doppler and myocardial performance index (MPI) as variables analyzed in patients with hepatitis C virus.

Therefore, this study was developed to evaluate new parameters in patients infected with the hepatitis C virus (HCV) when the disease is chronic and in its initial stages.

Methods

Study design - Control-case observational study initiated in September, 2000, and concluded in July, 2003. The project was submitted to the Comissão de Ética em Pesquisa da Instituição (*Institution Research Ethical Committee*) and was approved.

Population - Thirty one patients with chronic hepatitis C (group I), both genders, were randomly studied. In this group there were eighteen females, aged 12 to 49 years, mean age 37 years, supposedly without structural cardiopathy. The HCV diagnoses were established between 1994 and 2002. Twenty control-cases (group II), both genders, were randomly studied. In this second group there were ten females, aged 21 to 46 years, mean age 34 years, coming from the institutional staff, without antecedents of risk factors for HCV and without evidence of cardiopathy. All patients of group I presented serology and molecular biological test (*polimerase chain reaction* – PCR) positive for HCV, with or without specific

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therapy initiated, yet in non-advanced chronic infection stage, which means without hepatic cirrhosis, hepatocellular carcinoma or accentuated hepatic disfunction confirmed by hepatic biopsy. Patients with diabetes mellitus, pregnancy or with consumptive chronic diseases were not included.

Patients of both groups were submitted to clinical and eletrocardiographic evaluation during inclusion phase. Doppler echocardiographic evaluation was conducted using a Hewlett-Packard, Sonos 2000, echocardiograph, with a 3.5-2.5 MHz transducer and photo register with a Sonny 890 printer. The exams were registered in 50 or 100 mm/s and the measurements taken accordingly to criteria adapted from the American Society of Echocardiography[9]. For each evaluated parameter, three measurements were taken. Two examiners made the assessments. The following measurable parameters were analyzed through M-mode imaging and tissue and pulsatile Doppler:

Left ventricle echocardiometric evaluation (Fig. 1-A) - Left ventricular diastolic diameter (LVDD), left ventricular systolic diameter (LVSD), interventricular septum diastolic thickening (IVSDT) and left ventricular posterior wall diastolic thickening (LVPWDT).

Left ventricular functionals - Ejection Fraction (EF) measured according to Teichholz method through M-mode imaging, left ventricular circumferential shortening (LVCS) and the myocardial performance index (MPI) of LV expressing diastolic and systolic functions of that cavity.

Doppler of the mitral flow (Fig. 1-B) - Mitral flow protodiastolic velocity (E), Mitral flow telediastolic velocity (A), mitral flow E/A ratio and mitral flow decelerating time (DT). The MPI was measured from mitral flow intervals and LV outlet (Fig. 1-C).

Lateral mitral annulus Tissue Doppler (Fig. 1-D) - Mitral annulus protodiastolic velocity (E'), mitral annulus telediastolic velocity (A') and mitral annulus systolic lateral velocity (S).

Since the beginning of the study, new tissue Doppler variables for evaluation of diastolic changes were used – last 23 cases – for measurement of mitral annulus tissue velocities E', A' e S.

Laboratorial Exams – a) MEIA (microparticle enzyme immunoassay) of third generation was performed with AxSYM equipment (Abbott Diagnostics, EUA). C22, C33c, NS4 and NS5 were the HCV antigens used to improve microparticles sensitivity through fluorescent revelation system; b) Third-generation commercial Immunoblot (Cambridge, Iotech, EUA) performed as supplemental test; c) Polymerase Chain Reaction (PCR) used "in house" methodology with the external primers 5' NC – "BIO CCC TGT GAG GAA CTW CTG TCT TCA CGC" e 5' NC – 2 "BIO GGT GCA CGG TCT ACG AGA CCT"; d) Genotyping was performed through reverse hybridization method using InnoLipa (Innogenetcs, Bélgica).

Histopathological parameters to hepatic biopsy - The histopathological analysis consisted of classifications concerning the stage of structural changes from 0 to 4 and concerning the periportal activity, from 0 to 4, according to criteria from the Sociedade Brasileira de Hepatologia (Brazilian Society of Hepatology) and Sociedade Brasileira de Patologia do Fígado[10] (Brazilian Society of Pathology of the Liver) which establish periportal activity: 0 = normal, 1 = inflammatory infiltrated in most of portal spaces without aggression upon the limitant plaque, pattern of the former persistent chronic hepatitis, 2 = mild piecemeal necrosis (PMN), former active chronic hepatitis (mild ACH), 3 = moderate PMN (moderate ACH), 4 = severe PMN (severe HCA).

The same way structural alteration is classified: 0 = normal, 1 = portal and periportal fibrous expansion, 2 = score 1 (+) periportal septa formation, 3 = score 2 (+) portocentral septa formation with occasional nodules and extensive predominance of lobular structure, 4 = cirrhosis.

Statistical treatment - Quantitative data were analysed through Student's t-test for comparison of means between groups and are presented in mean values ± standard deviation, $p < 0,05$ chance probability and significance of 95% and Wilcoxon non-parametric test for interobserver concordance.

Results

The statistical analysis of the population sampling did not differ significantly in relation to mean age and gender distribution between both groups, as well as in relation to echocardiographic parameters obtained with M-mode echocardiogram or Doppler of the mitral flow or tissue Doppler of the mitral annulus (Tab. 1). The quantitative variables measured by Doppler on mitral flow spectrum and on left ventricle outlet of patients with HCV did not evidence significantly different results from those in the control group.

Hepatic histopathology revealed in all patients the stage of structural alteration ≤ 3 and periportal activity ≤ 2, indicating non-advanced stages of the disease (Tab. 2).

Discussion

The study results involving analysis of Doppler echocardiographic variables like thickening, cavity diameters and parameters of left ventricular systolic and diastolic functions as well as variables of Doppler of the mitral flow and Doppler tissue of the lateral mitral annulus are in accordance with some results related up to the present time, and corroborate that there would not be sufficient evidences to attribute to HCV a potential of aggression to human myocardium. Mitral flow E, A parameters, E/A ratio and DT, which analyze diastolic function, as well as the MPI useful in the analysis of LV[11] systodiastolic function did not show statistically significant differences in relation to the control group. In a study about HCV prevalence in patients with cardiomyopathies, performed in nineteen Japanese institutions, a 10.6% rate of HCV seropositivity was also found among patients with HCM, and 6.3% among patients with DCM in contrast to 2.4% in blood donors, and 5.4% in frequenters of five general hospitals[12].

Nevertheless, there are studies with a greater number of cases showing discordant results. Matsumori et al related a casuistry of ten HCV cases (13.5%) among 76 patients with DCM, indicating causal relation equally significant to the presence[13] of HCM. Afterwards, the same researchers brought out the results of a nine year follow-up in which fourteen patients (16.3%) were infected with HCV among 86 patients

with HCM[13]. Both studies used serological and molecular biology methods, as well as endomyocardial biopsy in its protocols. These findings were reinforced by Okabe et al[14] study with HCV viral material in myocardial tissue, as well as in the livers of three patients with myocarditis who died of congestive cardiac failure.

There are also investigations which indicate the lack of relation between HCV and cardiomyopathy development. In this way, in Greece, Dalekos et al.[15] followed 102 cases of patients chronically infected with HCV during 9.2 years, and 55 cases of patients with DCM during 13.3 months with diagnoses for HCV in blood samples, as well as for cardiomyopathy, although without viral histological or biomolecular analysis of myocardium tissue. In these groups it was not found causality correlation, neither in the manifestation of HCV in DCM carriers, nor in the detection of myocardial involvement in patients with HCV. However, the criteria for myocardial involvement anticipated stages not so incipient of left ventricular impairment (ejection fraction $\leq$ 45%), LVCS < 30% on M-mode, and LVDD > 57 mm/m^2, with or without symptoms. The authors suggested that ethnical or genetic hereditary factors, and others like habits and popular therapies, such as acupuncture and cutaneous incisions with non-sterilized knifes, could explain prevalence differences[15], although possible methodological differences and size sample would answer for the divergences found. Therefore, they concluded recommending neither the need of serological HCV tests in DCM carriers, nor follow-ups of HCV carriers viewing cardiomyopathy detection. In another important study conducted in Italy[16] 752 cardiopathy patients, enrolled in a cardiac transplant program of six hospitals, among them 309 with DCM, were researched in relation to HCV presence. The results showed serological positivity and global molecular biology of 5.4%. However, for the same aspect, the rate was 3.9% in the group with DCM, and 6.5% for non-myocardiopathy patients. The authors concluded that there was not causal relation between HCV and DCM.

Therefore, from these observations it was confirmed that the former studies focused on the analysis of HCV prevalence through serology and/or molecular biology in cardiomyopathy patients as research strategy of causal relation between that virosis and possible cardiac impairment. Japanese authors made the same with myocardial tissue from biopsy and necropsis material[17].

The present study was conducted with subjects infected with HCV and proved without cardiopathy, to evaluate left ventricular morphofunctional changes. Also more restrictive limitant criteria were defined for myocardial involvement

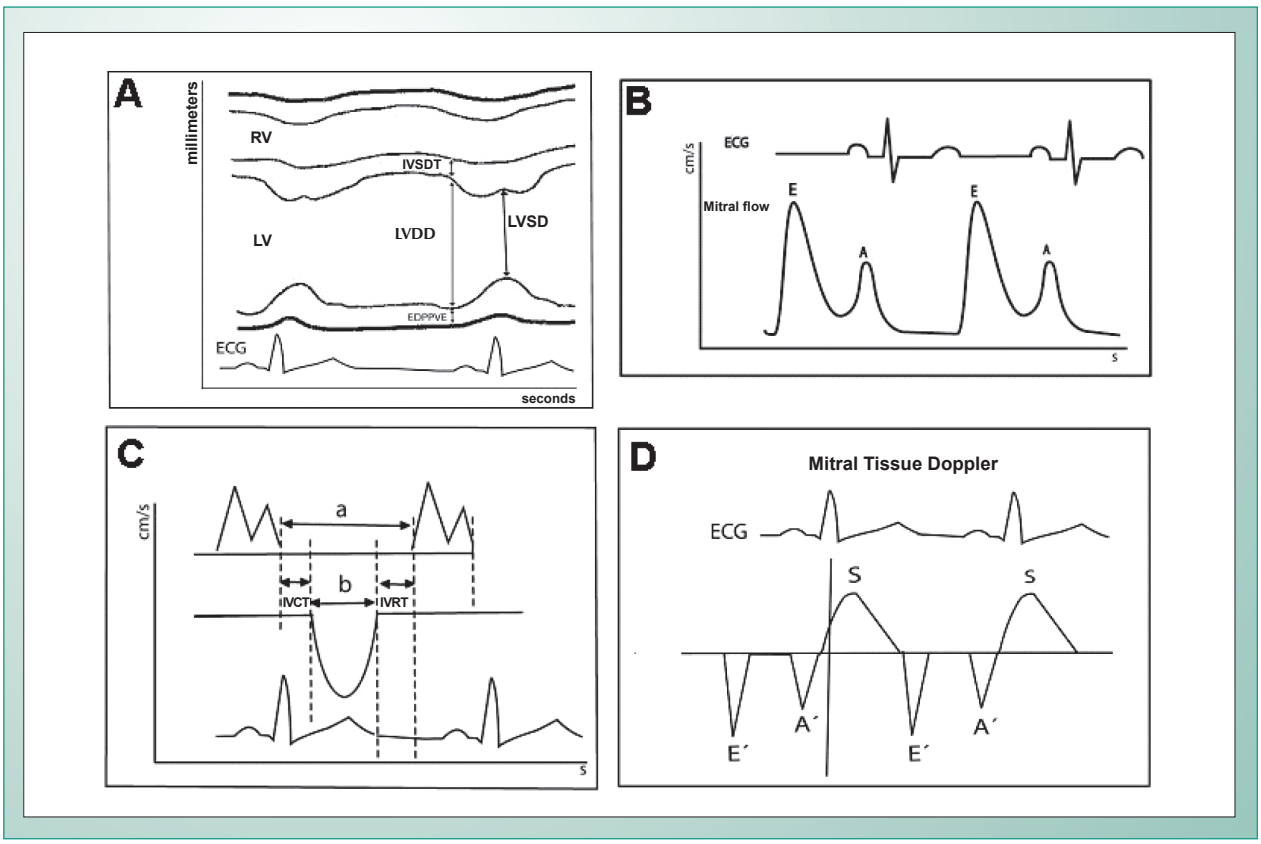

Fig. 1 - *Methodological diagram of evaluation of Doppler echocardiographic parameters used in the study. Methodology used in the study to evaluate Doppler left ventricular echocardiographic parameters. In A, thickenings and diameters measurements; in B, mitral flow velocity measurements; in C, spectral curve of the outlet; and in D, mitral annulus tissue velocities. Abbreviations: RV = right ventricular. LV = left ventricular. LVDD = left ventricular diastolic diameter. LVSD = left ventricular systolic diameter. ISDT = interventricular septum diastolic thickening. LVPWDT = LV posterior wall diastolic thickening. E = mitral transvalvular flow protodiastolic velocity. A = mitral transvalvular flow telediastolic velocity; MPI = myocardial performance index = (a - b / b) or (IVCT + IVRT/ ET), IVCT = isovolumetric contraction time. IVRT = isovolumetric relaxation time. ET = LV ejection time; E´= mitral annular protodiastolic velocity using tissue Doppler. A´ = mitral annulus telediastolic velocity using tissue Doppler. S = mitral annulus systolic velocity using tissue Doppler.*

	Group I (n = 31)	Group II (n = 20)	p value
Age (years)	12-49 (mean 37)	21-46 (mean 34)	NS
Female sex	18 (58%)	10 (50%)	NS
Normal ECG	54.8%	75%	NS
LAD (mm)	31.58±4.93	30.01±3.98	NS
LVDD (mm)	48.62±4.53	47.72±3.72	NS
LVSD (mm)	30.32±3.44	30.68±2.56	NS
ISDT (mm)	8.93±1.31	8.56±0.82	NS
LVPWDT (mm)	8.56±1.10	8.28±0.92	NS
LVCS (%)	37.61±4.48	35.84±2.27	NS
ET (ms)	314.97±21.93	303.46±22.50	NS
EF	0.67±0.06	0.68±0.04	NS
MPI	0.35±0.11	0.34±0.13	NS
E	76.87±15.09	73.01±14.07	NS
A	48.93±9.92	47.87±5.84	NS
E/A	1.62±0.39	1.53±0.30	NS
DT	184.58±24.34	178.48±21.81	NS
E´	15.21±3.68	15.87±2.52	NS
A´	10.93±2.27	11.22±2.12	NS
S	12.42±1.28	12.24±1.58	NS

ECG = electrocardiogram; LAD = left atrial diameter; LVDD = left ventricular diastolic diameter; LVSD = left ventricular systolic diameter; DIST = diastolic and interventricular septum thickening; LVPWDT = left ventricular wall diastolic thickening; LVCS = left ventricular circumferential shortening maximum diameter; ET = left ventricular ejective time; EF = left ventricular ejection fraction; MPI = myocardial performance index; E = mitral protodiastolic velocity; A = mitral telediastolic velocity; E/A = mitral E/A rate; DT = mitral flow deceleration time; E' = mitral annulus tissue protodiastolic velocity; A' = mitral annulus tissue telediastolic velocity; S = mitral annulus tissue systolic velocity. NS = statistically non-significant.

Table 1 - Parameters of both groups of patients, represented by values ± standard deviation

Grade	Structural alterations	Periportal activity
0	2 (6.5%)	1 (3.2%)
1	13 (41.9%)	13 (41.9%)
2	13 (41.9%)	17 (54.8%)
3	3 (9.7%)	0 (0%)
4	0 (0%)	0 (0%)

Table 2 - Histopathological hepatic profile of group I patients submitted to biopsy (n = 31)

which, in spite of their being potentially more sensitive, did not succeed in detecting causal relation between HCV and morphological and functional myocardial changes.

The tissue Doppler E', A' e S variables, which respectively reflect the proto and telediastolic *performance*, as well as the LV global contractile function were also normal and indistinguishable from those of the control group. In relation to the last, the mitral annulus systolic velocity S, the variable had its importance increased in the evaluation of the LV global contractile function, for quantitative measurements of the segmentary or global contraction were not performed. In the same way, these tissue variables were not used in the other studies analyzed.

Therefore, as the other studies were conducted with non-homogeneous populations and with cardiopathy patients, which can induce to equivocal conclusions, the findings of the present study suggest that cardiac impairment in patients infected with HCV is very questionable, mainly in a non-advanced stage of the disease.

Study limitations - One study limitation was the small casuistry of 31 HCV carriers on account of methodological difficulties, as obtaining permission to perform biopsies. Tissue Doppler was used only with the last 23 patients. However, as it was observed, all Doppler echocardiographic parameters didn't show differences between individuals of group I and group II, so it is reasonable to suppose that increasing the number of patients studied would not alter the results. Segmentary contractility quantitative measurements were not performed, for M-mode measurements were chosen, and the visual analyses did not indicate any abnormal contractility.

References

1. Tremolada F, Casarin C, Alberti A, et al. Long-term follow-up of non A, non B (type C) pos-transfusion hepatitis. J Hepatol 1992; 16: 273-81.
2. Alter MJ, Margolis HS, Krawczinsky K, et al. The natural history of community-acquired hepatitis C in the United States. N Engl J Med 1992; 327; 1899-905.
3. Alter, HJ. Descartes before the horse: I clone, therefore I am: The Hepatitis C virus in Current Perspective. Ann Intern Med 1991; 115: 644-9.
4. Ferreira C, Silveira T. Hepatites virais: aspectos da epidemiologia. Rev Bras Epid 2004; 7: 473-87.
5. Kiyosawa K, Tanaka E, Sodeyama T, Furuta S. Natural history of hepatitis C. Intervirology 1994; 37: 101-7.
6. Matsumori A, Yoshiki M, Sasayama S. Dilated cardiomyopathy associated with hepatitis C virus infection. Circulation 1995; 92(9): 2519-25.
7. Matsumori A, Matoba Y, Nishio R, Ono K, Sasayama S. Detection of hepatitis C virus RNA from the heart of patients with hypertrophic cardiomyopathy. Biochem Biophys Res Commun 1996; 222(3): 678-82.
8. Waggoner AD, Bierg SM. Tissue Doppler Imaging: A useful echocardiograph method for the cardiac sonographer to assess systolic and diastolic ventricular function. J Am Soc Echocardiogr 2001; 14(12): 1143-52.
9. Devereux RB, Lutas EM, Casale PN. Standardization of M mode echocardiographic left ventricular anatomic measurements. J Am Coll Cardiol 1984; 4: 1222-30.
10. Gayotto LCR. Comitê SBP/SBH. Visão histórica e consenso nacional sobre a classificação das hepatites crônicas. GED. Gastrenterol Endosc Dig 2000; 19(3): 137-40.
11. Salgado AA, Albanesi Filho FM, Castier M, Bedirien R. Índice de performance miocárdica. Fim da fração de ejeção? Rev Bras Ecocardiog 2004; 17(3): 69-74.
12. Matsumori A, Osashi N, Hasegawa K, et al. Hepatitis C virus infection and heart diseases: a multicenter study in Japan. Circ J Journal 1998; 62: 389-91.
13. Matsumori A. Symposium on clinical aspects in hepatitis virus infection. Clinical practice of hepatitis: myocardial diseases, nephritis, and vasculitis with hepatitis C virus. Intern Med 2001; 40: 182-4.
14. Okabe M, Fukuda K, Arahawa K, Kikuchi M. Chronic variant of myocarditis associated with Hepatitis C virus infection. Circulation 1997; 96: 22-4.
15. Dalekos GN, Achenbach K, Christodoulou D, et al. Idiopathic dilated cardiomyopathy: lack of association with hepatitis C virus infection. Heart 1998; 80: 270-5.
16. Prati D, Poli F, Farma E, et al. Multicenter study on hepatitis C virus infection in patients with dilated cardiomyopathy. J Med Virol 1999; 58: 116-20.
17. Matsumori A, Yutani C, Ikeda Y, Kawai S, Sasayama S. Hepatitis C virus from the hearts of patients with myocarditis and cardiomyopathy. Mod Pathol 2000; 80(7): 1137-42.