Effects of Hepatitis C virus on cardiovascular risk in infected patients: A comparative study

C.P.M.S. Oliveira a, C.R. Kappel b, E.R. Siqueira c, V.M.R. Lima a, J.T. Stefano a, M.T. Michalczuk b, S.S. Marini b, H.V. Barbeiro d, F.G. Soriano d, F.J. Carrilho a, L.M.M.B. Pereira e, M.R. Alvares-da-Silva b, c, * a University of Sao Paulo School of Medicine, Department of Gastroenterology (LIM07), Sao Paulo, Brazil b Universidade Federal do Rio Grande do Sul, Gastroenterology Division of Hospital de Clinicas de Porto Alegre, School of Medicine, Porto Alegre, Brazil c Liver Institute of Pernambuco, Recife, Brazil d University of Sao Paulo School of Medicine, Department of Emergency (LIM51), Sao Paulo, Brazil

A B S T R A C T

The role of hepatitis C virus (HCV) in the pathogenesis of atherosclerosis and cardiovascular events is unclear. The aim of this study was to evaluate the direct effect of HCV on cardiovascular risk and correlate it with pro and anti-inflammatory cytokines in patients with HCV. HCV monoinfected patients, genotype 1, naive, non-obese (BMI < 30) and non-diabetics were included and compared to controls (blood donors). Patients with prior diagnosis of cardiovascular diseases, hypertension, chronic renal failure, cancer and chronic use of lipid-lowering drugs or immunosuppressants were excluded. Serum cytokines (IL-6, IL-10 and TNF-α) and Framingham score were also evaluated. 62 HCV patients, 34 (54.8%) were males and none of them was smoking. The Framingham scores (median and 25th and 75th percentiles) were 12% (6.5–14%), showing an intermediate cardiovascular risk in patients with HCV. There was significant direct correlation between Framingham and total cholesterol (p = 0.043) and DBP (p = 0.007). HDL-C (p = 0.002) was inversely correlated with the Framingham score. HCV patients had higher levels of proinflammatory cytokines (IL-6 and TNF-α) compared to controls (p < 0.0001) and the relation of proinflammatory/anti-inflammatory TNF-α/IL10 and IL-6/IL10 were higher in HCV patients (p < 0.01). The Framingham score was directly correlated to IL-6 and TNF-α, but differences were not statistically significant. Patients with HCV monoinfected, nonobese, naive and non diabetic have an intermediate cardiovascular risk, as measured by the Framingham score and high levels of proinflammatory cytokines (IL-6 and TNF).

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1. Introduction

Hepatitis C virus (HCV) infects about 2–3% of the world population [1] and approximately 4 million of Americans are estimated to have been infected with HCV [2]. HCV infection leads to chronic hepatitis in up to 60–80% of infected individuals and is associated with liver steatosis, fibrosis and, insulin resistance (IR) [3–6]. Furthermore, some studies have demonstrated an association between infectious diseases such as Chlamydia, Helicobacter pylori and also HCV and coronary atherosclerosis and carotid artery plaque [7–11]. HCV infection is recognized to cause chronic immune stimulation, leading to an inflammatory response and cytokine production [12,13]. Two distinct patterns of cytokines production may occur. Type 1 responses are characterized by production of IL-2, TNF-α and IFN-γ, which prime and maintain antigen–specific cellular immunity and are important in defense against viruses. Type 2 responses are characterized by IL-4, IL-5, IL6- and IL-10, which promote humoral immune responses [14]. These altered cytokine profiles observed in the setting of chronic HCV could potentially lead to adverse cardiovascular outcomes. Thus, HCV induces inflammatory cytokines, with consequent increase in intracellular adhesion molecules, expression of anti-endothelium antibodies and generation of oxidative stress, produces IR, interferes with the lipid metabolism, and is associated to Diabetes mellitus type 2 (DM) and systemic vasculitis. Due to the above-mentioned reasons, it is easy to suppose that HCV would be able to increase the cardiovascular risk [10].

Nevertheless, HCV is associated with a favourable lipid profile, with lower cholesterol and LDL levels [15]. A retrospective evaluation
of a predominantly genotype 4 Egyptian cohort found that patients with chronic hepatitis C infection had significantly lower levels of LDL cholesterol and triglycerides when compared to those who had never been infected with hepatitis C [16,17]. This hyperlipidemia resolves with successful hepatitis C treatment but persists in nonresponders [18]. Thus, it is unknown how infection with HCV affects coronary heart disease (CHD) risk, progression and outcomes [19].

Based on these prior findings, the aim of this study was to evaluate the direct effect of HCV on cardiovascular risk and correlate it with serum concentrations of pro and anti-inflammatory cytokines in patients with HCV.

2. Patients and methods

2.1. Population

HCV mono-infected patients, naive, non-obese [Body mass index (BMI) below 30], with no clinical signs of cirrhosis, non-diabetic, aged between 18 and 60 years, from an outpatient clinic of Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil and Liver Institute of Pernambuco, Recife, Brazil, were included and compared to controls. This HCV group consisted of 62 subjects defined as documented positive HCV antibody and detectable HCV RNA, all of them submitted to liver biopsy. Those with human immunodeficiency virus and/or hepatitis B coinfection, hemochromatosis or recipients of solid organ transplants were excluded. Subjects with clinical evidence of cirrhosis, determined by the presence of portal hypertension, defined by esophageal or gastric varices on endoscopy, ascites or splenomegaly or evidence of synthetic dysfunction on laboratory evaluation were also excluded. The controls were consecutive blood donors from the Blood Bank of the Clinic Hospital of University of São Paulo non-infected with HCV or other viruses, without metabolic syndrome factors (dyslipidemia, DM, high blood pressure, central obesity), and with an IMC under 30. They had their clinical and biochemical parameters registered.

Age, BMI, systolic blood pressure (SBP) and diastolic (DBP), fasting glucose and lipid levels were determined. Identification of type 2 DM, hypertension and dyslipidemia followed the recommendations of the American Diabetes Association [20].

Patients with prior diagnosis of cardiovascular diseases, systemic blood hypertension, chronic renal failure, cancer, alcohol abuse, pregnancy and chronic use of lipid-lowering drugs or immunosuppressants were excluded.

2.2. Laboratory evaluation and serum cytokine measurement

The laboratory evaluation in all patients included a blood cell count and the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), g-glutamyltranspeptidase, total cholesterol and fractions, triglycerides, fasting glucose, and insulin levels. These parameters were measured using the standard techniques of clinical chemistry laboratories (Modular P800, Hitachi, Roche Applied Science, Indianapolis, IN, USA). IR was measured by the HOMA-IR index (Chicago, IL, 148 USA) was employed.

For the cytokine and chemokine measurements, the serum was stored at −80°C until use. The serum cytokine levels (TNF-α, IL-6, and IL-10) were then measured using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D System Inc, Minneapolis, MN). All measurements were made in duplicate, and the average values were used in the statistical analyses.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCV (n = 62)</th>
<th>Controls (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean ± SD</td>
<td>52.94 ± 8.55</td>
<td>51.2 ± 9.31</td>
<td>NS</td>
</tr>
<tr>
<td>Sex % male/female</td>
<td>54.8/45.2</td>
<td>53.4/46.7</td>
<td>NS</td>
</tr>
<tr>
<td>BMI &gt;30</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Tobacco</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrosis – METAVIR n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 &amp; F2 (12.9)</td>
<td>37 (59.7)</td>
<td>F2 &amp; F3 (16.1)</td>
<td>ND</td>
</tr>
<tr>
<td>F4 (11.3)</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Total cholesterol mg/dL mean ± SD</td>
<td>152.26 ± 26.8</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>HDL cholesterol mg/dL mean ± SD</td>
<td>49.6 ± 15.9</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>FRS 10 yr median (25–75th percentiles)</td>
<td>12 (6.5–14)</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>HOMA-IR median (25–75th percentiles)</td>
<td>1.94 (1.51–3.48)</td>
<td>ND</td>
<td>NA</td>
</tr>
</tbody>
</table>

Where: HCV = hepatitis C virus; SD = standard-deviation; NS = non-significative; ND = non-determined; NA = not applied; FRS = Framingham risk score; HOMA-IR = Homeostasis Model Assessment For Insulin Resistance.

2.3. Framingham Risk Scoring System (FRS)

The 10-yr likelihood for development of cardiovascular events was estimated for each subject using the multivariate scoring system of the Framingham Heart Study [21,22]. It calculates the risk from age, sex, systolic blood pressure, the ratio of total to high-density lipoprotein (HDL) cholesterol, smoking habit, and presence of diabetes in subjects between the ages of 45 and 75 yr. Patients at risk to undergo myocardial infarction within the next 10 yr, according to the FRS were stratified into low (10%), intermediate (10–20%), and high (20%) midterm risk groups.

3. Histological analysis

The liver tissue was fixed in 4% formaldehyde and processed for hematoxylin–eosin and Masson trichrome stains for histological analysis. Histological analyses were evaluated by pathologist who was unaware of the HCV genotype as well as the patient’s clinical characteristics. Stages of fibrosis and grades of inflammation were scored according to METAVIR, that it consists of F0 (no fibrosis), F1 (portal fibrosis without septa), F2 (portal fibrosis with few septa), F3 (numerous septa without cirrhosis), F4 (cirrhosis). Steatosis was graded 0–3 based on percentages of hepatocytes harbouring lipid droplets in the biopsy (0 reflecting none; 1 equalling 0–33%; 2 referring to 33–66%; and 3 representing >66% steatotic hepatocytes).

3.1. Ethical consent

Specific informed consent was obtained for the study and the protocol was approved by the Internal Review Board of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, Liver Institute of Pernambuco, Recife, Brazil, and University of São Paulo, São Paulo, Brazil. The study was conducted in conformance with the Helsinki Declaration. All patients gave written informed consent.

3.2. Statistical analysis

Student’s t test and Wilcoxon test were used according to normality assessment (Kolmogorov–Smirnov). By the same token, linear regression analysis (Pearson or Spearman correlation) was selected as appropriate, complemented with logistic regression for qualitative variables (gender and diagnosis). SPSS for Windows, version 10.0 (Chicago, IL, 148 USA) was employed. p values of less than 0.05 were considered significant.

4. Results

Demographic and clinical features of the subjects (n = 62) and control are listed in Table 1. 62 patients HCV, 28 (45.2%) were female and 34 (54.8%) males were evaluated. None of them was smoking. The
Framingham scores (median and 25th and 75th percentiles) were 12% (6.5–14%), showing an intermediate cardiovascular risk in patients with HCV. There was significant direct correlation as evaluated by the Spearman test between Framingham and total cholesterol (p = 0.043) and DBP (p = 0.007). HDL-C (p = –0.002) was inversely correlated with the Framingham score. HCV patients had higher levels of proinflammatory cytokines (IL-6 and TNF-α) compared to controls – Figs. 1 and 2, but there was no difference between the groups regarding IL-10. The Framingham score was directly correlated to IL-6 (Spearman rho = 0.120) and TNF-α (Spearman rho = 0.306), and inversely to IL-10 (Spearman rho = –0.013), but differences were not statistically significant. The relation of proinflammatory/anti-inflammatory TNF-α/IL10 and IL-6/IL10 were higher in HCV patients (p<0.01) – Fig. 3. There was no correlation among IR and Framingham score or cytokine profile.

5. Discussion

Currently, cardiovascular risk in HCV patients has been an interesting focus, and the relationship between cardiovascular risk, lipids, cytokines and atherosclerosis in these patients is not completely elucidated. In the present study, we demonstrated that monoinfected, non-obese, non-diabetic and non-clinically cirrhotic HCV patients, have an intermediate cardiovascular risk, as measured by the Framingham score (FRS) and high levels of proinflammatory cytokines (IL-6 and TNF).

Cytokines play an important role in the defense against viral infections, both indirectly, through determination of the predominant pattern of host response, and directly, through inhibition of viral replication. However, in the context of an inflammatory response against a virus, cytokines may also lead to liver damage.

There are some recently published studies focusing on cardiovascular disease during chronic HCV infection. Two of them are particularly interesting, and deserve some comments. In the first one, the Heart and Soul Study [23], a cohort of more than 900 patients with cardiovascular disease was evaluated – 8.6% of them HCV positive. Those infected with the virus have higher TNFα as well as higher risk of cardiac failure and death. The second, a study from Egypt [24], with a large number of patients, has demonstrated that patients infected with HCV has higher intima-media thickness than controls. However, we consider that the main issue is to verify the relationship between HCV and cardiovascular risk, not cardiovascular disease, as the first is more prone to be able to respond to clinical intervention.

Regarding cardiovascular risk assessment, there are some applicable tools, as the determination of C-reactive protein, proinflammatory cytokines, FRS and also computed tomography-coronary artery calcium score. Some of them are employed to evaluate HCV-related disease progression. In Table 2, the comparison between the present and previous published studies on cardiovascular risk assessment tools in HCV patients is shown.

In the current study, we observed higher levels of proinflammatory cytokines (IL-6 and TNF-α) in HCV patients compared to controls. Besides, we described by the first time that the relation of proinflammatory/anti-inflammatory TNF-α/IL10 and IL-6/IL10 were higher in HCV patients. These findings are very important because there are some studies demonstrating that most liver-infiltrating T cells in chronic hepatitis C are type 1-like cytokines (TH1), such as IL-1b, IL-2, IL-6, IL-8, TNF-α, and IFN-γ are upregulated in chronic HCV [25]. However, few studies have been drawn to the role of anti-inflammatory cytokine levels. Interleukin 10 (IL-10) is a potent anti-inflammatory Th2 cytokine that down-regulates the expression of major histocompatibility complex (MHC) class I and class II molecules, as well as the production of Th1 cytokines [26–31]. How proinflammatory/anti-inflammatory cytokines relations could interfere in the pathogenesis of atherosclerosis in Chronic HCV infection is very intriguing and unclear. We just hypothesized according to our results that when these relations (TNF-α/IL10 and IL-6/IL10) were higher in HCV patients the cardiovascular risk could be increased as measured by FRS. In this setting, a recent epidemiologic study showed that HCV-seropositive blood donors had higher rates of cardiovascular mortality compared to uninfected donors and the altered cytokine profiles observed in the setting of chronic HCV could potentially lead to adverse cardiovascular outcomes [32,33].

Another important factor related to the cytokine profile that could contribute to a increased cardiovascular risk in HCV-infected patients...
is the concurrent higher IR than the observed in patients with other chronic liver diseases, mainly due the association with activation of TNF-α system and high IL-6 levels [34]. Lecube et al. demonstrated that proinflammatory cytokines in HCV positive patients with chronic hepatitis without diabetes and without advanced liver fibrosis have increased levels of sTNFR1, sTNTR2 and TNF-α and these cytokines have relationship with HOMA-IR suggesting that is a mechanism by which HCV-infected patients are more prone to develop type 2 diabetes than patients with other chronic liver diseases [34]. The relation between inflammation and IR in the pathogenesis of type 2 diabetes is well established [34].

![Graph showing levels of IL-6 in controls and patients with hepatitis C (HCV).](image)

**Fig. 2.** Levels of IL-6 in controls and patients with hepatitis C (HCV).

![ROC curve of data showing TNFα/IL - 10 and IL - 6/IL - 10.](image)

**Fig. 3.** ROC curve of pro-inflammatory/anti-inflammatory indexes in patients with hepatitis C in comparison to controls. AUC = area under curve; IC = interval of confidence; S = sensibility; E = specificity.
DM and HCV had been suggested in some years ago [35]. The mechanisms by which HCV impairs insulin sensitivity include increased TNF-α and IL-6 production, over-expression of suppressor of cytokines signal 3 (SOCS-3), as they interfere with the intracellular signalling pathway of insulin [36]. Our study didn’t show this association among IL-6 levels and IR.

Recently, Milner et al. [37] demonstrated that the HCV-induced IR is mainly related to the muscle; it is also independent of increased hepatic lipid (in genotype 3). Moreover, peripheral IR in hepatitis C was unusually associated with subcutaneous fat, suggesting a possible interplay between the virus and the adipocyte, which will need to be further examined [37].

Our findings are consistent with other studies finding low circulating cholesterol and LDL levels in patients with chronic HCV infection. However, when considering the FRS, the overall percentage of HCV patients with intermediate or high 10-yr likelihood for development of CHD was 1.5-fold increased compared with the general population. In fact, similar results have been described by Corey at al [18] who demonstrated that the serum lipids are low because lipids play a role in HCV virion circulation and hepatocyte entry. Besides, these authors observed that the hypolipidemia resolves with successful HCV treatment but persists in nonresponders. A significant proportion of successfully treated patients experience LDL and cholesterol rebound to levels associated with increased coronary disease risk. This intriguing paradox between lower serum lipids and FRS, associated with higher inflammatory and pro-antinflammatory rates of cytokines in our study, could suggest that chronic inflammation in HCV infection leads to modulation of cytokines Th1/Th2 such as TNF-α and IL-6 increasing the systemic inflammatory cascade without increasing the lipid levels, however predisposing to atherosclerosis and cardiovascular risk independently of serum lipids levels. Ruan et al. [38] demonstrated that inflammatory stress changes the phenotype of LDLr regulation from sensitive to resistant for cholesterol-mediated downregulation An increased threshold for LDL uptake in liver and peripheral cells may lower plasma cholesterol. This could be a mechanism for the paradoxical association of low cholesterol with cardiovascular disease in some patients that have chronic inflammation [38]. Thus, may be that long-term chronic inflammation disrupts the sensitivity of feedback regulation in peripheral cells, therefore, LDL cholesterol is not only transported to the liver, but also to the peripheral tissues with impaired cholesterol efflux under inflammatory stress which cause excess cholesterol accumulation, foam cell formation, and low LDL cholesterol concentration [39]. Interestingly, Chen et al. [39] demonstrated that IL-1β causes statin resistance in both hepatic cell line (HepG2) and human kidney mesangial cells (HMCs) under inflammatory conditions [38]; therefore, higher concentrations of statin may be required to achieve the same degree of biological effect in each cell type. It may also explain why statins, which provide cardiovascular protection in the general population, do not reduce cardiovascular mortality in dialysis patients with DM in chronic inflammatory states [40].

In summary, even in patients previously expected to have low-risk to cardiovascular diseases, HCV seems to increase that risk, as the presence of the infection was related to higher FRS as well as higher pro-inflammatory cytokine profile.

Acknowledgement

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

References


Table 2
Factors related to the assessment of cardiovascular risk and its application in populations of HCV infected patients. Comparison between the present study and others previously published.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiovascular risk*</th>
<th>Disease progression</th>
<th>Present study**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Evaluated Result</td>
<td>Evaluated Result</td>
<td>Evaluated Result</td>
</tr>
<tr>
<td>CRP</td>
<td>Yes</td>
<td>Increased</td>
<td>Yes</td>
</tr>
<tr>
<td>IL-6</td>
<td>No</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>TNFα</td>
<td>Yes</td>
<td>Increased</td>
<td>No</td>
</tr>
<tr>
<td>Pro/Anti</td>
<td>No</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>FRS</td>
<td>No</td>
<td>NA</td>
<td>No</td>
</tr>
</tbody>
</table>

Where: * non-selected HCV population (naïve and non-naïve, cirrhotic and non-cirrhotic, obese and non-obese, diabetics and non-diabetics); ** selected HCV patients; Evaluated means that the variable was studied in order to determine a definite endpoint (cardiovascular risk or disease progression); Result means if the variable was related to the endpoint studied; the present study endpoint was cardiovascular risk; CRP = C-reactive protein; Pro/Anti = Pro/Anti-antiinflammatory rates; FRS = Framingham risk score.


