Atherosclerosis 221 (2012) 496–502

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Chronic HCV infection is a risk of atherosclerosis. Role of HCV and HCV-related steatosis

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ARTICLE INFO

Article history:
Received 22 September 2011
Received in revised form
30 December 2011
Accepted 31 January 2012
Available online 8 February 2012

Keywords:
HCV
Carotid atherosclerosis
Liver steatosis
HOMA-IR
Metabolic syndrome
Homocysteinemia

ABSTRACT

Objectives: HCV and NAFLD are associated with atherosclerosis in general population. The prevalence of atherosclerosis in chronic hepatitis C (CHC) patients is unknown. We hypothesized that HCV per se and HCV-related steatosis could favour atherosclerosis. Thus, in CHC patients we assessed: (a) the prevalence of atherosclerosis; (b) the role of HCV, cardio-metabolic risk factors and hepatic histology.
Methods: Overall, 803 subjects were enrolled: (A) 326 patients with liver biopsy-proven treatment naive CHC (175 with and 151 without steatosis); (B) 477 age and gender matched controls, including 292 healthy subjects without steatosis (B1) and 185 with NAFLD (B2). Carotid atherosclerosis (CA), assessed by high-resolution B-mode ultrasonography, was categorized as either intima-media thickness (IMT: >1 mm) or plaques (≥1.5 mm).
Results: CHC patients had a higher prevalence of CA than controls (53.7% vs 34.3%; $p < 0.0001$). Younger CHC (<50 years) had a higher prevalence of CA than controls (34.0% vs 16.0%; $p < 0.04$). CHC patients without steatosis had a higher prevalence of CA than B1 controls (26.0% vs 14.8%; $p < 0.02$). CHC with steatosis had a higher prevalence of CA than NAFLD patients (77.7% vs 57.8%, $p < 0.0001$). Viral load was associated with serum CRP and fibrinogen levels; steatosis with metabolic syndrome, HOMA-IR, hyperhomocysteinemia and liver fibrosis. Viral load and steatosis were independently associated with CA. Diabetes and metabolic syndrome were associated with plaques.
Conclusion: HCV infection is a risk factor for earlier and facilitated occurrence of CA via viral load and steatosis which modulate atherogenic factors such as inflammation and dysmetabolic milieu.

1. Introduction

Hepatitis C virus (HCV) infection is endemic worldwide, with an estimated global prevalence of 3%, resulting in more than 170–200 million infected people [1].

Several infectious agents have been associated with coronary artery disease. Earlier studies on the general population showed that HCV markers were independently associated with atherosclerosis [2,3]. Subsequent research, however, yielded conflicting results; some confirming [4,5] and others denying such an association [6,7]. Recently, HCV RNA sequences have been isolated within carotid plaques, supporting the hypothesis of a direct pro-atherogenic role of HCV [8]. However, the prevalence of atherosclerosis in chronic HCV infected patients is unknown.

Patients with non-alcoholic fatty liver diseases (NAFLD) show a high prevalence of atherosclerosis [9–11]. Hepatic steatosis is a feature of chronic hepatitis C (CHC) [12,13]. HCV-related steatosis is associated with many pro-atherogenic factors, such as insulin resistance (IR) [13], hyperhomocysteinemia [14], cytokine imbalance [15], oxidative stress [16], overweight/obesity [17] and diabetes [18].

We hypothesized that HCV per se and HCV-related steatosis are associated with atherosclerosis in CHC patients. Accordingly, we assessed the prevalence of carotid atherosclerosis (CA) in a cohort of CHC patients with and without steatosis compared with a control group. Furthermore, the role of HCV, cardio-metabolic risk factors and liver histology as determinants of CA were also evaluated.

Abbreviations: CHC, chronic hepatitis C; IMT, carotid intima-media thickness; HOMA-IR, homeostatic model assessment; IR, insulin resistance; HCV, hepatitis C virus; HCV RNA, hepatitis C virus RNA; HAI, histological activity index; CRP, C reactive protein.

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0021-9150/$ – see front matter © 2012 Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2012.01.051

2. Materials and methods

2.1. Study design

This prospective study compares data from consecutive CHC patients (group A) with and without steatosis and a non-HCV group age- and gender-matched control (group B) from the same geographic area. Group B included two subgroups: healthy subjects without steatosis (group B1) and NAFLD patients (group B2). Group B included also patients with type 2 diabetes and hypertension with and without steatosis in a number equivalent to that observed in group A. All diagnostic procedures (liver biopsy, ultrasonography, laboratory analysis) and serum stored (−30 °C) were done at same time point, at time of liver biopsy.

Based on epidemiological studies, we estimated that the difference in the prevalence of atherosclerosis was as follows: at least 20% between all those with CHC and controls, and at least 10% between CHC without steatosis and respective control. To test our hypothesis, for a power of 0.80, on the basis of an error $\alpha = 0.05$ and a supposed prevalence difference (Δ) of p1–p2 = 20 or 10, the calculated sample size for CHC population was of at least 93 cases and for HCV without steatosis was of at least 142 patients for a two-sided test. HCV and controls were matched 1:2.

The study protocol was conform to 1975 Declaration of Helsinki and approved by Ethic Committee and informed consent were obtained by all participants.

2.2. Patients

2.2.1. Group A: HCV patients

Treatment naïve Italian patients admitted to our clinics from 2005 to 2011 for diagnostic work-up of CHC were enrolled. Patients HBV/HIV positive, alcohol (≥40 g/die) or drug abusers, those with oesophageal varices, decompensated cirrhosis, and proteinuric renal disease were excluded. No patients received either steatogenic drugs or aspirin/statins.

2.2.2. Group B: control group

Given that HCV patients were quite heterogeneous as far as steatosis and cardiovascular risk factors are concerned, we recruited controls both without (B1) and with steatosis (B2) and CV risk factors.

Subgroup B1. Included healthy subjects recruited amongst HCV patients' relatives, sanitary personnel and general population screened for routine check up. Inclusion criteria were HCV-abs and HBsAg negativity; absence of steatosis at liver ultrasound scanning; no overt disease; routine haematological, renal and metabolic (except for cholesterol) laboratory profile within the normal range; no medications. Before evaluation for atherosclerosis, subjects, were age- and gender-matched at ratio of 2:1 with HCV infected patients without steatosis.

Subgroup B2. Included NAFLD patients. The diagnosis of NAFLD was based on the following criteria: (a) absent or low alcohol intake (<30 g daily for man and <20 g for woman); (b) presence of steatosis at ultrasound scanning; and (c) absence of alternative etiologies of liver disease, notably viral, autoimmune, thyroid, drug-induced, hemodynamic and genetic-metabolic. Liver biopsy was proposed whenever indicated. This group was compared with CHC patients with steatosis.

All subjects were submitted to an accurate recording of clinical history, signs and symptoms of cardiovascular disease and to ECG. Echocardiography was done in all hypertensive subjects and in those with a history of heart disease. Previous diagnosis and/or current ischemic heart disease were a reason for exclusion.

2.3. Parameters evaluated

Smokers were categorised as past-smokers and current smokers. The number of cigarettes smoked per day was also recorded.

BMI (kg/m^2) and waist circumference were evaluated; visceral obesity was defined as a waist circumference >102 (males) and >88 cm (females).

A full panel of hepatic and renal laboratory tests was obtained in all patients. Moreover, the following tests were performed: blood cell count, C-reactive protein (CRP), fibrinogen, total serum cholesterol, triglycerides, HDL and LDL cholesterol, plasma glucose, serum insulin.

Plasma homocysteine was determined by fluorescence polarization immune-assay (IMX, Abbott Laboratories, Abbot Park, IL, USA).

Serum insulin was assayed by a radioimmunoassay method (Insulin RIA DSL-1600, Diagnostic System Laboratories, USA). IR was evaluated by HOMA-IR using the following formula: [fasting plasma glucose (mmol/l) × fasting serum insulin (μIU/ml)]/22.5. The cut-off value for normal HOMA-IR was the 75th percentile of the value obtained for 120 "healthy" sex- and age-matched subjects from the same geographical area [19]. In the "healthy" group, the value of HOMA-IR was 1.97 and the cut-off level of HOMA-IR was established at 2.6.

Metabolic syndrome was diagnosed following the ATP III criteria [20]. Hypertension was defined as a blood pressure >130 mmHg and diastolic >85 mmHg.

Diabetes was diagnosed in presence of treatment or with fasting blood glucose ≥126 mg/dl in two different days. Impaired fasting plasma glucose (IFPG) was defined by fasting glucose in the 110–125 mg/dl range.

HCV-RNA positivity was evaluated by Amplicor HCV (Roche Diagnostics); viral load was evaluated by real time PCR (Monitor HCV-Amplicor; Roche Diagnostics) and viral genotyping by Inno-Lipa HCV assay (Bayer). HBV and HIV markers and autoimmunity were explored using commercially available kits.

2.4. Liver histological evaluation

Liver biopsy was performed under ultrasound assistance. Necroinflammatory activity was assessed by Histology Activity Index (HAI), and fibrosis stage was scored according to Ishak [21]. Steatosis was graded as follows: 0: absent to ≤5% of hepatocytes; 1: 6–30%; 2: 31–60%, 3 >60% of hepatocytes. The pathologist was blind to patients' data.

2.5. CA evaluation

Arterial wall stiffness and carotid intima-media thickness (IMT), a well-accepted, easy, noninvasive and reproducible method of assessing subclinical atherosclerosis burden, were assessed by high-resolution B-mode ultrasonography (Esaote Techos, Genova, Italy) equipped with a 7.5 MHz linear-array transducer. Semi-automatic IMT measurements were taken at both carotid arteries at the levels of the common (CCA), internal (ICA) and bulb tracts. IMT was measured as the difference between the first (intima-lumen) interface and the second (media-adventitia) interface on the far wall of the artery. IMT measurements of the carotid artery (common, bulb, and internal) were taken in triplicate for each site and the mean value was calculated and recorded. In agreement with the Mannheim Carotid Intima-Media Thickness Consensus [22], IMT >1 mm was regarded as a cut-off value for carotid atherosclerosis (CA) given that it is associated with a 3–4-fold increased risk of subsequent ischemic stroke [22]. Moreover, IMT has been indicated as a powerful predictor of coronary further to cerebrovascular events [22–28]. Plaques were defined as protrusions into the vessel