The risk of subclinical carotid atherosclerosis in patients with chronic hepatitis C

Le risque d’athérosclérose carotidienne infra clinique chez les patients atteints d’hépatite C chronique

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Summary

Background: The role of hepatitis C virus in the pathogenesis of atherosclerotic disease has been suggested by several studies. We aimed to assess the association between subclinical carotid atherosclerosis and chronic hepatitis C.

Methods: 40 patients infected with chronic hepatitis C and 40 control cases were evaluated by anthropometric and metabolic measurements. The risk of subclinical atherosclerosis was assessed by ultrasound measurement of carotid intima-media thickness. A high cardiovascular risk atherosclerosis was defined by carotid intima-media thickness > 75th percentile.

Results: The carotid intima-media thickness and the prevalence of high cardiovascular risk atherosclerosis were significantly higher in the group infected with hepatitis C compared to the control group (0.68 VS 0.60, p=0.02) and (82.5% vs 40%, 0.001) respectively. In multivariate studies, activity ≥ A2 and age > 40 years were the independent factors associated with the carotid intima-media carotid and hepatitis C was the only independent factor associated with high cardiovascular risk atherosclerosis (OR = 4.81 at 95%: 1.6 to 14.42).

Conclusions: In our study, chronic hepatitis C was associated with a high risk of carotid atherosclerosis.

Key-words: Chronic hepatitis C, atherosclerosis, carotid intima-media, Insulin resistance, cardiovascular risk.
INTRODUCTION
The prevalence of hepatitis C virus (HCV) worldwide is estimated at 1% (1). HCV is thought to be involved in insulin resistance (IR), diabetes and in atherosclerotic disease(2,3).

HCV is associated with several atherogenic conditions such as IR (4), steatosis (5) and chronic inflammation (3). Some studies have also shown that HCV replicates in carotid plaques causing vascular inflammation (6,7).

Measurement of carotid intima-media thickness (CIMT) with B-mode ultrasound is a sensitive technique for identifying atherosclerotic burden and cardiovascular disease (CVD) risk (8,9).

In our study, we evaluated the risk of carotid subclinical atherosclerosis in patients with chronic hepatitis C, compared with a control group.

METHODS
Our study was conducted from March 2015 to June 2017. In all, 40 patients with chronic hepatitis C (CHC) were prospectively recruited at the hepato-gastro-enterology department of Mohamed Taher Maamouri hospital. The inclusion criteria were: the presence of anti-HCV and HCV RNA without any features of acute hepatitis C. All patients met the following exclusion criteria: 1) Age ≤ 18 years, 2) cirrhosis Child Pugh B or C , 3) Hepatocellular carcinoma, 4) associated chronic liver disease: alcoholic hepatitis, hepatitis B, autoimmune hepatitis, primary biliary cholangitis, Wilson’s disease, hemochromatosis, alpha antitrypsin deficiency, 5) alcohol consumption> 30g / day in men and> 20g / day in women, 6) co-infection with human immunodeficiency virus, 7) the use of steatosis-inducing drugs (corticosteroid, tamoxifen, amiodarone) or drugs interfering with lipid metabolism (statin, fibrate), 8) previous hepatitis C anti-viral treatment , 9) previous history of symptomatic atherosclerotic disease, 10) illicit drug addiction.

Forty patients, matched for sex, age, and body mass index (BMI) with the CHC group, were enrolled as controls. They were recruited at our department and suffered from gastroesophageal reflux disease and irritable bowel syndrome. All of them had negative anti-HCV and met the exclusion criteria noted above.

Clinical and biological data:
Clinical, anthropological and biological data were collected at the time of inclusion.

The BMI was calculated according to the following formula: 
BMI = weight (kg) / height (m²). Patients were classified as obese if BMI > 30 kg / m².

Biochemical analyses were performed in the same central laboratory for all patients, including cholesterol (CT), HDL-cholesterol (HDL-CT), triglycerides (TG), glycemia and insulinemia.

Insulin resistance (IR) was evaluated by the index of homeostasis model assessment of insulin resistance (HOMA-IR) (10).

The criteria of the International Diabetes Federation (11) were adopted for the definition of the metabolic syndrome (MS).

Virological data:
The viral load of HCV (HCV –RNA) was measured by quantitative PCR in real time and expressed in IU/ml. The viral genotype C was determined by the viral sequencing method.

Evaluation of chronic hepatitis C fibrosis :
The inflammatory necrotic activity of hepatitis C was determined with fibrotest actitest test (12). It was performed only in patients with no clinical and biological signs of cirrhosis. Fibrosis was considered significant if ≥ F2.

Evaluation of the risk of atherosclerosis:
The CIMT was evaluated by an expert radiologist, using a high-resolution B-mode ultrasonography equipped with a multifrequency linear probe (8,9). CIMT was measured manually, at the left and right distal common carotid artery at 1 cm from the carotid bifurcation, on a segment measuring at least 10 mm. The CIMT maximum (CIMT max) was defined as the highest CIMT measurements from the left and right side.

The diagnosis of Infra-clinical atherosclerosis with a high cardiovascular risk was based on CIMT max > 75th percentile (CIMT 75)(8,13).
Statistical study

The statistical analysis and data processing were performed with SPSS software (SPSS for Windows, version 22).

The Continuous variables, expressed on average ± standard deviation, were compared with Student t-test. The categorical variables, expressed on frequency, were compared with the chi 2 test and the exact test of Ficher.

To investigate factors associated with infra-clinical atherosclerosis, a univariate study and a multivariate study were conducted with the dependent variables CIMT\textsuperscript{max} and CIMT\textsuperscript{75}.

For the univariate study, we used the ANOVA test for qualitative variables and bivariate correlation for quantitative variables. Differences were considered statistically significant if \( p < 0.05 \). The correlation was classified as low (Bravais-Pearson r coefficient is close to 0), strong (\( r \) approaches +1 or -1) and no correlation (\( r = 0 \)).

Variables associated with the dependent variable at univariate analysis (\( P < 0.10 \)) were included in the multivariate regression models: linear regression model for the dependent variable CIMT\textsuperscript{max} and the binary logistic regression model for the dependent variable CIMT\textsuperscript{75} were used. The associations were expressed as odds ratios (OR) with their confidence intervals (95% CI).

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices and with local and national laws.

RESULTS

The distribution of baseline characteristics of the two groups were detailed in Table 1. The mean age of the CHC patients was 55±15.9 years. Twenty-six CHC patients were over 50 years. The two groups were comparable for age, sex, BMI, co-morbidities, smoking, and anthropological data.

Insulinemia and HOMA-IR index were significantly higher in the CHC group (\( p = 0.005 \) and \( p = 0.001 \) respectively). The CT and HDL-CT were significantly lower in the CHC group (\( p = <0.001 \) and \( p = 0.012 \) respectively). Fifteen patients and 6 controls had a MS (37.5% vs. 15%, \( p = 0.02 \)).

The predominant genotype OF HCV was 1 (92.5%). Significant fibrosis was noted in 27 patients (67.5%) and 15 patients (37.5%) had severe fibrosis (F3/4).

The mean value of CIMT\textsuperscript{max} was significantly higher in the CHC group compared to the control group (0.68 VS 0.60 mm, \( p = 0.02 \)). The prevalence of CIMT\textsuperscript{75} was significantly higher in the CHC group compared to the control group (82.5% vs 40%, \( p < 0.001 \)).

Factors associated with the CIMT\textsuperscript{max}

CIMT\textsuperscript{max} was weakly correlated with age (\( r = 0.48, p < 0.001 \)) but significantly increased in age ≥ 40 years (0.67 VS 0.47 mm, \( p < 0.001 \)). HCV infection was associated with higher values of the CIMT\textsuperscript{max} (Table 1). Significant fibrosis was associated with a higher CIMT\textsuperscript{max} in the CHC group. The value of the CIMT\textsuperscript{max} was comparable between the control and CHC group with non-significant fibrosis (fig n°1). For necrotico-inflammatory activity, stage ≥ A2 was associated with a higher CIMT\textsuperscript{max} (0.76 VS 0.61 mm, \( p = 0.004 \)).

![Figure 1. The CIMT max in CHC group and control group](image)

Patients with fibrosis < F2 had a mean CIMT max value comparable to the control group, but significantly lower than patients with fibrosis ≥ F2

The HOMA -IR index was weakly correlated with the CIMT\textsuperscript{max} (\( r = 0.31, p = 0.005 \)). On the other hand, there was no association between CIMT\textsuperscript{max}, the SM (\( p = 0.17 \)) and IR (\( p = 0.5 \)).
Table 1. Baseline demographic, laboratory, and metabolic features in hepatitis C group and control group

| Variables                          | CHC* group (n=40) | Control group (n=40) | p   |
|------------------------------------|-------------------|----------------------|-----|
| Mean age, (years)                  | 55 ±15,9          | 51,9 ±12,46          | 0,26|
| Gender (H/F)                       | 13/27             | 17/23                | 0,36|
| diabetes (N/%)                     | 7(17,5%)          | 3(7,5%)              | 0,18|
| Arterial hypertension (N/%)        | 10 (25%)          | 15(37,5%)            | 0,22|
| dyslipidemia (N/%)                 | 0                 | 1(2,5%)              | -   |
| Smoking (N%)                       | 9(22,5%)          | 6(15%)               | 0,57|
| BMI * (Kg/m²)                      | 27,26 ±5,15       | 26,37±4,51           | 0,42|
| Obesity                            | 12(30%)           | 9(22,5%)             | 0,55|
| Glycemia (mmol/l)                  | 6,2 ± 2,33        | 5,21±0,53            | 0,012|
| Insulinemia µUI/ml                 | 12,93 ± 6,35      | 9,38 ±4,62           | 0,005|
| HOMA –IR index                     | 3,6±2,28          | 2,16±1,07            | 0,001|
| IR*                                | 27 (67,5%)        | 15(37,5%)            | 0,007|
| CT* (µmol/l)                       | 3,76 ± 0,99       | 4,91±0,99            | <0,001|
| HDL-CT* (µmol/l)                   | 1,24 ± 0,33       | 1,45±0,38            | 0,012|
| TG* (µmol/l)                       | 0,96 ± 0,29       | 1,07±0,5             | 0,215|
| MS * (%)                           | 15 (37,5%)        | 6(15%)               | 0,02|
| CIMT max * (mm)                    | 0,68 ± 0,16       | 0,60±0,13            | 0,002|
| CIMT75 *                            | 33 (82,5%)        | 16 (40%)             | < 0,001|
| HCV-RNA* UI/ml                     | 3 697 572         | 6043422,57           | -   |
| Genotype 1/2                       | 37/3              | -                    | -   |
| Stage of fibrosis                  | F0                | 3 (7,5%)             | -   |
| F1                                 | 10(25%)           | -                    | -   |
| F2                                 | 12(30%)           | -                    | -   |
| F3                                 | 6(15%)            | -                    | -   |
| F4                                 | 9(22,5%)          | -                    | -   |
| Grade of inflammation              | A0                | 6 (15%)              | -   |
| A1                                 | 13 (32,5%)        | -                    | -   |
| A2                                 | 12 (30%)          | -                    | -   |
| A3                                 | 6 (15%)           | -                    | -   |
| Not determined                     | 3 (7,5%)          | -                    | -   |
| Ultrasound steatosis               | 15 (37,5%)        | -                    | -   |

*CHC : chronic hepatitic C; *BMI : body mass index ;IR* :insulinoresistance ;HOMA* : homeostasis model assessment; CT* : cholesterol ; HDL* : high density lipoprotein acid ;TG* : triglycerides ; MS * : metabolic syndrom ; CIMT* : carotid intima-media thickness ; CIMT75* : carotid intima-media thickness ≥ 75 percentiles ; HCV-RNA* : hepatitis C virus ribonucleic .

In a multi-variate study, only age ≥ 40 years and moderate-to-severe activity of hepatic impairment were independent factors associated with higher CIMTmax (p<0.001 and p=0.03 respectively).

**Factors associated with infra clinic atherosclerosis with high cardiovascular risk**

In univariate studies, the factors associated with the CIMT75 were HCV infection (p<0.0001), HOMA-IR index (p = 0.001), IR (p = 0.004) (Table 2). In multivariate analysis, only HCV infection was identified as an independent factor associated with CIMT75 (OR=4.81, CI :1,6-14,42, p=0.005) (Table 2).

**DISCUSSION**

In our study, it was shown that patients with chronic hepatitis C had a CIMTmax significantly higher compared to the control group. HCV infection has also been identified as the only independent factor associated with subclinical atherosclerosis with high cardiovascular risk.

Conflicting literature data have reported either normal (14-17) or increased CIMT (6,18-21), in patients with a clinical diagnosis of HCV infection compared with control populations.

Ishizaka et al were the first to report an association between carotid atherosclerosis and HCV infection, in a cohort of 1992 Japanese subjects. HCV-infected patients had a higher CIMT compared to non-infected subjects, as well as a significantly higher prevalence of carotid plaques (64% vs 25%, p<0.0001) (18).

These data were supported by a meta-analysis including nine case-control studies (22), chronic HCV infection was significantly associated with a higher CIMT compared to control subjects (p<0.001).

Other studies have noted a negative association between HCV infection and atherosclerosis. Most of them have included a small number of HCV-infected patients. Bilora et al followed the progression of atherosclerotic disease in 40 HCV-infected subjects and 40 controls for 5 years, both groups were matched for the cardiovascular risk factors and had a baseline comparable CIMT (14). A significant increase in CIMT was only observed in the control group.

Similarly, Tien et al found that HCV infection was associated neither with the CIMT nor with the presence of...
carotid plaques (16). The same data were confirmed by an Egyptian study (17).

Few studies have evaluated the impact of fibrosis in chronic hepatitis C on the CIMT. They showed that severe fibrosis was associated with a higher risk of carotid atherosclerosis (20,21).

In our work, significant fibrosis and moderate to severe activity were associated with a higher value of IMT on univariate study.

In the literature, no studies have directly evaluated the association between chronic HCV infection and subclinical atherosclerosis with high cardiovascular risk. The studies were rather focused on the impact of HCV infection on CVD mortality.

In the meta-analysis of Petta et al, HCV-infected patients had increased risks of CVD-related mortality compared with uninfected individuals (OR :1.65; CI 95%, 1.07-2.56; p=0.022). In an American cohort including more than 10,000 HCV-infected blood donors, HCV infection was associated with an increased risk of cardiovascular mortality (OR=2.21, 95% CI: 1.41, 3.46) (23).

In a 16-year prospective cohort study, HCV infection was associated with increased hepatic and extra-hepatic mortality. CVD mortality was significantly higher in patients with detectable viral RNA (HR = 2.77, 95 % CI 1.49-5.15) (24).

The pathogenic mechanisms explaining the role of HCV in atherogenesis are not fully understood. Chronic hepatitis

Table 2. Univariate and multivariate analysis of risk factors associated with a high cardiovascular risk atherosclerosis

| Variables       | CIMT <75 (-) | CIMT <75 (+) | uni-variate study p | multi-variate study OR ( CI 95%) | p   |
|-----------------|--------------|--------------|---------------------|---------------------------------|-----|
| HCV(+)          | 17.5%        | 82.5%        | <0.001              | 4.81 (1.6-14.42)/0.005           |     |
| Mean age (years)| 50.38±14.75  | 55.89±13.8   | 0.86                | -                               |     |
| Age > 40 years  | 31.2%        | 43.7%        | 0.14                | -                               |     |
| Gender          | 17.5%        | 20%          | 0.26                | -                               |     |
| Diabetes        | 3.75%        | 8.75%        | 0.55                | -                               |     |
| Arterial hypertension | 12.5%    | 18.75%       | 0.90                | -                               |     |
| Smoking         | 8.75%        | 11.25%       | 0.65                | -                               |     |
| Obesity         |              | 0.27         | -                   | -                               |     |
| CT *mmol/l      | 4.7±0.94     | 4.1±1.21     | 0.40                | -                               |     |
| HDL-C * µmol/l | 1.34±0.38    | 1.35±0.38    | 0.66                | -                               |     |
| TG * µmol/l    | 0.97±0.46    | 1.03±0.38    | 0.50                | -                               |     |
| Glycemia mmol/l| 5.23±0.67    | 6.0±2.13     | 0.65                | -                               |     |
| Insulinemia µU/ml | 8.7±3.66 | 12.7±6.39    | 0.43                | -                               |     |
| HOMA-IR*        | 2.01±0.84    | 3.43±2.19    | 0.001               | 1.66 (0.71-3.84)/0.24           |     |
| IR*             | 12.5%        | 40%          | 0.004               | 0.94 (0.15-5.97)/0.95           |     |
| MS*             | 7.5%         | 18.75%       | 0.27                | -                               |     |
| HCV-RNA* UI/l  | 3244375,2±4256499,65 | 3793704,6±640662377 | 0.83 | - |
| Fibrosis ≥ F2   | 10%          | 22.5%        | 0.13                | -                               |     |

*CIMT>75%*: carotid intima-media thickness>75 percentiles ; *OR : odds ratio ; *CI : confidence interval ; *HCV : hepatitis C virus ; *CT : cholesterol ; *HDL : high density lipoprotein acid ; *TG, triglycerides ; *HOMA, :homeostasis model assessment ; *IR :insulinoresistance ; *MS :metabolic syndrom ; * HCV-RNA : hepatitis C virus ribonucleic .
C is associated with a complex condition promoting pro-atherogenic factors that are involved in endothelial dysfunction, insulin resistance, fibrosis and steatosis (9,25). These factors would be interconnected and driven mainly by dysregulation of several actors of innate and adaptive immunity (26,27).

Moreover, HCV RNA sequences have been detected in carotid plaques and in cerebral endothelium, suggesting a probable direct pro-atherogenic role of HCV (6,7,28).

The main limitation of our study lies in the small number of patients included and a selection bias in the recruitment of the control population which could affect the interpretation of results. The strength of our study lies in the fact that we did not only assess the CIMT as available data from literature but we were interested also in the evaluation of carotid atherosclerosis at high cardiovascular risk with a direct impact on cardiovascular disease.

CONCLUSION

Our findings suggest that the HVC was associated with an increased risk of Infra-clinical atherosclerosis with a high cardiovascular risk and we proposed that patients with chronic hepatitis C should receive periodic cardiovascular evaluation regardless of the presence of conventional cardiovascular risk factors.

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