

## Prospective Study

## Naïve hepatitis B e antigen-negative chronic hepatitis B patients are at risk of carotid atherosclerosis: A prospective study

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## Abstract

### BACKGROUND

There is an increased risk of atherosclerosis in patients with chronic hepatitis C or human immunodeficiency virus, but there is scarce data on hepatitis B virus infection. The hypothesis of this study is that hepatitis B virus infection increases the risk of carotid plaques and subclinical atherosclerosis in naïve hepatitis B e antigen (HBeAg) negative subjects.

### AIM

To assess the rate of carotid plaques and subclinical atherosclerosis in naïve HBeAg negative subjects in comparison with a cohort of healthy controls.

### METHODS

Prospective case-control collaborative study conducted in two tertiary hospitals. Four hundred and two subjects prospectively recruited at the outpatient clinic were included from May 2016 to April 2017: 201 naïve HBeAg-negative hepatitis

were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. It was approved by the Ethics Committee of both hospitals (PR(AG)245/2015).

**Informed consent statement:**

Informed verbal consent was obtained from all individual participants included in the study and recorded at the medical records.

**Conflict-of-interest statement:**

Riveiro-Barciela M has received research grants from Gilead and served as speaker for Gilead and Grifols. Esteban R has received research grants from Gilead and has served as advisors for Gilead, Bristol-Myers Squibb and Novartis. Buti M has received research grants from Gilead and has served as advisors for Gilead, Bristol-Myers Squibb and Novartis. The rest of authors have no personal or financial conflicts of interest.

**Data sharing statement:** Technical appendix, and dataset available from the corresponding author at [[mbuti@vhebron.net](mailto:mbuti@vhebron.net)]. Participants gave informed verbal consent for data sharing.

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B virus-infected [49 chronic hepatitis B (CHB) and 152 inactive carriers(ICs)] and 201 healthy controls. Anthropomorphic and metabolic measures, liver stiffness and carotid Doppler ultrasound were performed. Subclinical atherosclerosis was established on an intima-media thickness increase of  $\geq 1.2$  mm and/or the presence of carotid plaques. Normally distributed quantitative variables were compared with the Student *t* test and those with a non-normal distribution with the Mann-Whitney *U* test. Categorical variables were compared between groups using the  $\chi^2$  or Fisher exact test.

**RESULTS**

Carotid plaques were found more often in CHB (32.7%) than ICs (17.1%) or controls (18.4%) ( $P = 0.048$ ). Subclinical atherosclerosis was also increased in CHB (40.8%) vs ICs (19.1%) or controls (19.4%) ( $P = 0.003$ ). No differences in the risk of atherosclerosis were observed between controls and ICs. The factors independently associated with the presence of carotid plaques were age [odds ratio(OR) 1.43,  $P < 0.001$ ] and CHB (OR 1.18,  $P = 0.004$ ) and for subclinical atherosclerosis, age (OR 1.45,  $P < 0.001$ ), CHB (OR 1.23,  $P < 0.001$ ) and diabetes (OR 1.13,  $P = 0.028$ ). In the subset of young subjects ( $< 50$  years), carotid plaques (12.5% vs 1.1%,  $P = 0.027$ ) and subclinical atherosclerosis (12.5% vs 2.2%,  $P = 0.058$ ) were more frequent among CHB than ICs.

**CONCLUSION**

Untreated HBeAg-negative CHB is an independent risk factor for carotid plaques and subclinical atherosclerosis, while ICs present a similar risk to controls.

**Key Words:** Hepatitis B virus; Carotid plaques; Subclinical atherosclerosis; Cardiovascular risk; Endothelial dysfunction; Intima-media thickness

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**Core Tip:** This prospective case-control collaborative study aimed to assess whether chronic infection by hepatitis B was associated with risk of carotid plaques and subclinical atherosclerosis. Overall, 402 subjects were recruited, 201 naïve hepatitis B e antigen-negative hepatitis B virus-infected and 201 healthy controls. Patients with hepatitis B e antigen-negative chronic hepatitis B presented a higher rate of carotid plaques than non-infected controls, but no differences were observed between controls and hepatitis B inactive carriers. These results suggest that hepatitis B infection may have a role as a cardiovascular risk factor in patients with chronic hepatitis B.

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**INTRODUCTION**

More than 257 million people worldwide are infected with hepatitis B virus (HBV)[1], and more than 780000 die each year due to the infection[2]. Chronic HBV infection is a dynamic condition that passes through several phases, being the hepatitis B e antigen (HBeAg)-negative form the most common in Western countries[1]. Currently, patients are classified as HBeAg-negative chronic hepatitis B (CHB) when they have increased HBV DNA and alanine aminotransferase (ALT) levels and liver fibrosis and/or necroinflammation or as HBeAg-negative chronic infection/inactive carriers (ICs) when they have low HBV DNA and normal ALT levels and associated with absent or mild liver damage[3].

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An increased risk of cardiovascular events has been associated with some viral infections like hepatitis C virus (HCV)[4] or human immunodeficiency virus (HIV)[5] as well as autoimmune diseases[6]. The cause of atherosclerosis in these patients is not fully explained by conventional risk factors, and endothelial dysfunction has been suggested as the underlying mechanism causing the early atherosclerotic process. This endothelial dysfunction is mainly associated with the persistent inflammatory state linked to these diseases (HCV, HIV and autoimmune diseases). In fact, eradication of HCV infection has shown a positive impact on carotid atherosclerosis[7]. Both the presence of carotid plaques or measurement of the intima-media thickness (IMT) are accepted and validated surrogate markers for early diagnosis of subclinical atherosclerosis leading to increased cardiovascular risk[8].

Chronic HBV infection has been associated with a propensity to mount proinflammatory immune reactions[9,10], including higher oxidative stress[11], that may predispose to a higher subclinical atherosclerosis.

The aim of this study was to assess whether the stage of HBeAg-negative chronic HBV infection impacts the presence of both carotid plaques and subclinical atherosclerosis. Another aim was to evaluate if the risk of both carotid plaques and subclinical atherosclerosis in HBeAg-negative patients differ to those of healthy controls.

## MATERIALS AND METHODS

### Patients

Two hundred and one patients with chronic HBV infection and naïve to antiviral therapy were prospectively recruited at the outpatient clinics of two tertiary hospitals (Di.Bi.M.I.S., University of Palermo, Italy and Vall d'Hebron Hospital, Spain) from May 2016 to April 2017. Inclusion criteria were hepatitis B surface antigen (HBsAg) positive for more than 6 mo, HBeAg-negative and no prior exposure to antiviral therapy. Exclusion criteria were previous cardiovascular events (acute myocardial infarction or ischemic stroke), liver transplantation, HCV, hepatitis D or HIV coinfection, history of hepatocellular carcinoma or evidence of liver disease of mixed etiology (autoimmune hepatitis, Wilson's disease, hemochromatosis,  $\alpha$ 1-antitrypsin deficiency). In addition, 201 healthy individuals matched for sex, age and body mass index were recruited as controls at the outpatient clinics from the same centers. In particular, no patient had a history of previous cardiovascular events, evidence of HBV infection (HBsAg and anti-HBc negative), HCV or HIV, or history of rheumatic or oncological disease. Importance of selection of naïve patients was crucial in view of the effect of antiviral therapy in both liver immunity and carotid plaques in subjects with HCV treated with direct-acting antivirals[7].

Naïve patients with HBV infection were classified into CHB and IC according to the recommendations of European Association for the Study of the Liver[3]: HBeAg-negative CHB was established on HBV DNA > 2000 IU/mL plus fluctuating or persistently elevated ALT levels and/or histological evidence of at least moderate fibrosis and/or necroinflammation; HBeAg-negative chronic infection or IC state was established on persistently normal ALT levels plus HBV DNA < 2000 IU/mL or HBV DNA 2000-20000 IU/mL plus evidence of mild or absent hepatic necroinflammation and fibrosis. Diagnosis of liver cirrhosis was established by liver biopsy (Ishak score 5 or 6) or transient elastography values > 13.1 kPa[12]. This study was conducted in accordance with the Declaration of Helsinki guidelines and the principles of Good Practice and was approved by the Ethics Committee of both hospitals (PR(AG) 245/2015).

### Baseline clinical and laboratory assessment

Data on demographics (sex, age and race), toxic exposure (alcohol, tobacco), cardiovascular risk factors (on-treatment arterial hypertension, diabetes and dyslipidemia) and anthropomorphic characteristics (height, weight, and waist circumference) were prospectively collected at the time of enrollment. A blood test was performed including hematology and a standard biochemical panel as well as insulin level, glycated hemoglobin, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, C-reactive protein, HBV serology (quantitative HBsAg, anti-HBc, HBeAg and anti-HBe) and HBV virology. HBV DNA was determined using the COBAS 6800 HBV test (Roche Diagnostics, Mannheim, Germany), with a lower limit of quantification of 20 IU/mL and lower limit of detection of 10 IU/mL. Antibodies against HCV, hepatitis D virus and HIV were also tested.

Central obesity was defined as a waist circumference greater than 102 cm in men and 88 cm in women. Insulin resistance was determined with the homeostasis model assessment[13]. The nonalcoholic fatty liver disease (NAFLD) score was also calculated, and values > 0.675 were considered suggestive of advanced NAFLD-related fibrosis[14]. Liver elastography (Fibroscan® 502 Touch, Echoscans, Paris, France), including the control attenuation parameter (CAP) as a marker to quantify hepatic steatosis, was carried out in all patients. CAP was chosen as marker of liver steatosis because it has been pointed out as more accurate than other scores such as Hepatic Steatosis Index in patients with chronic infection by HBV[15].

### **Carotid artery evaluation**

Carotid Doppler ultrasound study (Vivid I, General Electric, GE Healthcare, Horten, Norway, equipped with a 3.5-10 MHz linear transducer) was performed to determine the IMT. B-mode ultrasound with a semi-automatic edge-detection algorithm was used to measure the medium and maximum IMT on the far wall of both the right and left side of the common carotid artery at 1 cm before the bifurcation, measuring at least 250 mm of a straight arterial segment. The presence of an atheroma plaque was established based on the Mannheim criteria, as a focal structure that encroached into the arterial lumen by at least 0.5 mm or 50% of the surrounding IMT value or demonstrated a thickness > 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface[16]. The presence of plaques was investigated in the common carotid artery and internal and external carotid arteries. Subclinical atherosclerosis was established on an increased IMT ( $\geq 1.2$  mm) and/or detection of a carotid plaque[16]. To avoid interobserver variability, ultrasound measurements were performed by operators specifically trained in carotid ultrasound cardiovascular risk assessment. Moreover, measurement of the IMT at the common carotid artery presented high reproducibility and interobserver agreement in previous multicenter studies[17].

### **Statistical analysis**

Normally distributed quantitative variables were compared with the Student *t* test and those with a non-normal distribution with the Mann-Whitney *U* test. Quantitative variables were expressed as the median and interquartile range or mean and standard deviation depending on the group size. Categorical variables were compared between groups using the  $\chi^2$  or Fisher exact test, as appropriate. Variables with a *P* value < 0.10 in the univariate model were analyzed in a multivariate logistic regression model. Quantitative variables were also introduced as categorical (median or mean of the overall cohort) in order to increase the potency of the models. In the case of homeostasis model assessment, values from included patients were contrasted with the normal from general population[18]. Odds ratios (ORs) and 95% confidence intervals were calculated for the independent predictive factors of carotid plaques and subclinical atherosclerosis. Only patients with available data for all the variables considered in the analysis were included in the multivariate logistic regression models.

Because enrollment of patients with CHB was difficult due to the limitation to naïve subjects, the number of CHB and ICs differed. For this reason, a propensity score analysis matched by sex, age and main cardiovascular risk factors was carried out by using the package of R[19]. *P* values < 0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS, version 26.0 (SPSS Inc, Armonk, NY, United States).

## **RESULTS**

### **Baseline characteristics of patients**

In total, 402 individuals were enrolled: 201 chronic HBV-infected and 201 healthy controls. Overall, 218 (54.2%) were males, and the more common cardiovascular risk factors were active or former smokers (33.3%), alcohol intake (25.8%) and dyslipidemia (19.9%). Both alcohol intake and central obesity were more common in the control group. **Table 1** shows the baseline characteristics of the included cohorts of patients.

In the HBV-infected group, 152 (75.6%) were ICs and 49 (24.4%) CHB. In the latter, 12 (24.4%) patients had liver cirrhosis. Baseline characteristics according to the classification of HBV infection were summarized in **Table 1**. Most patients were Caucasian (68.2%), and the median age was 47 years. Demographical features did not differ between the two groups. Dyslipidemia was more common in ICs than in patients with CHB, whereas the prevalence of the remaining cardiovascular risk factors was similar. ALT, HBV DNA and HBsAg values as well as liver stiffness were significantly higher

**Table 1** Baseline characteristics of included subjects and comparison between infected and non-infected subjects and among patients infected by hepatitis B virus according to the phase of the infection (chronic hepatitis B vs inactive carriers)

	Controls	Chronic hepatitis B	Inactive carriers	P value <sup>1</sup>	P value <sup>2</sup>
	n = 201	n = 49	n = 152		
Age, yr	48.1 ± 10.2	48.4 ± 12.0	46.5 ± 13.4	0.29	0.28
Male sex (%)	103 (51.2)	31 (64.6)	84 (54.9)	0.13	0.16
Race (%)				< 0.001	0.48
Caucasian	186 (92.5)	35 (72.9)	102 (66.7)		
Asian	2 (1.0)	6 (12.5)	11 (7.2)		
African	0 (0)	6 (12.5)	28 (18.3)		
Hispanic	13 (6.5)	1 (2.1)	12 (7.8)		
Cardiovascular risk factors (%)					
Tobacco exposure	74 (36.8)	15 (31.3)	45 (29.6)	0.09	0.48
Alcohol intake <sup>3</sup>	70 (34.8)	11 (22.9)	23 (15.4)	< 0.001	0.17
Hypertension	40 (19.9)	11 (22.9)	27 (17.8)	0.46	0.28
Diabetes	4 (2.0)	4 (8.3)	6 (3.9)	0.08	0.2
Dyslipidemia	46 (22.9)	3 (6.3)	31 (20.4)	0.08	0.02
Central obesity	52 (25.9)	10 (20.8)	27 (17.9)	0.04	0.4
BMI, kg/m <sup>2</sup>	25.3 ± 3.6	26.0 ± 3.9	25.2 ± 4.0	0.76	0.22
Liver cirrhosis (%)	0 (0)	12 (24.4)	0 (0)	< 0.001	< 0.001
ALT, IU/mL	22.6 ± 12.7	59.7 ± 48.6	25.6 ± 16.7	< 0.001	< 0.001
GGT, IU/mL	30.4 ± 30.9	60.7 ± 87.9	31.5 ± 63.3	0.24	< 0.001
LDL, mg/dL <sup>4</sup>	131.9 ± 38.3	116.9 ± 30.7	118.1 ± 32.6	0.002	0.82
Triglycerides, mg/dL	108.0 ± 56.7	96.4 ± 46.3	106.8 ± 59.1	0.54	0.26
C-reactive protein, mg/dL <sup>5</sup>	0.29 ± 0.42	0.84 ± 1.90	1.00 ± 9.00	0.42	0.88
HOMA index <sup>5</sup>	2.05 ± 1.84	4.20 ± 3.50	3.40 ± 3.90	< 0.001	0.18
HBsAg, logIU/mL	-	3.6 ± 0.8	2.9 ± 1.2	-	0.001
HBV DNA, logIU/mL	-	4.4 ± 1.8	2.4 ± 1.1	-	< 0.001
Transient elastography, kPa	4.5 ± 1.4	11.3 ± 10.9	5.5 ± 2.4	< 0.001	< 0.001
CAP, dB/m	246.5 ± 54.5	227.4 ± 55.0	227.2 ± 56.2	0.001	0.98

Data are expressed as the median (interquartile range) or as the *n* (%).

<sup>1</sup>Comparison between hepatitis B virus-infected and non-infected controls.

<sup>2</sup>Comparison between patients with chronic hepatitis B and inactive carriers.

<sup>3</sup>Significant alcohol intake was defined as > 30 g per day for men and > 20 g per day for women.

<sup>4</sup>These data were available in 132 non-infected subjects.

<sup>5</sup>This data was available in 83 non-infected subjects. ALT: Alanine transaminase; CAP: Control attenuation parameter; GGT: Gamma glutamyltransferase; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; BMI: Body mass index; LDL: Low-density lipoprotein; HOMA: Homeostasis model assessment.

in patients with CHB.

### **Carotid plaques and subclinical atherosclerosis in HBV-infected group in comparison with the control group**

No differences were observed between the HBV-infected group and the control group in terms of gender and age, although some cardiovascular risk factors such as central obesity and dyslipidemia were more common among non-HBV infected individuals (Table 1). In fact, although increased values of liver stiffness were observed in patients with HBV infection, CAP levels were higher in subjects within the control group.



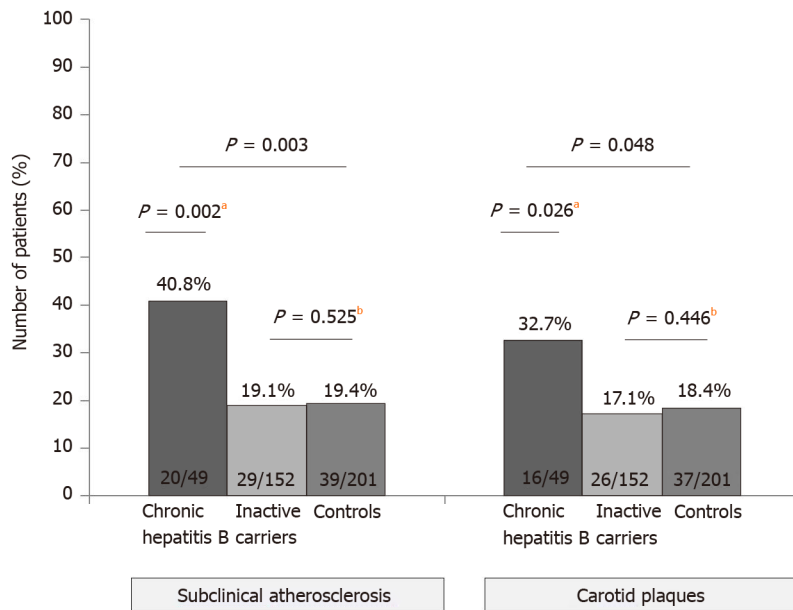
**Table 2** Factors associated with the presence of carotid plaques and subclinical atherosclerosis

	Subclinical atherosclerosis			Carotid plaques		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
		OR (95%CI)	P value		OR (95%CI)	P value
Age, years	< 0.001			< 0.001		
Age > 50 years	< 0.001	1.45 (1.24-1.48)	< 0.001	< 0.001	1.43 (1.21-1.44)	< 0.001
Male sex	0.004		0.336	0.003		0.212
Central obesity	0.008		0.073	0.007		0.141
Tobacco exposure	0.003		0.081	0.002		0.187
Alcohol intake <sup>1</sup>	0.109		-	0.073		0.929
Arterial hypertension	< 0.001		0.949	< 0.001		0.690
Diabetes mellitus	0.004	1.13 (1.03-1.59)	0.028	0.01		0.082
Dyslipidemia	0.001			< 0.001		0.095
Chronic hepatitis B	0.001	1.23 (1.11-1.41)	< 0.001	0.016	1.18 (1.06-1.34)	0.004
Transient elastography, kPa	0.01		0.090	0.073		0.438
Transient elastography > 5.7 kPa	0.008			0.048		
CAP, dB/m	< 0.001		0.989	< 0.001		0.577
CAP > 238 dB/m	< 0.001			< 0.001		
AST, IU/mL	0.115		-	0.152		-
AST > 27 IU/mL	0.102			0.131		
GGT, IU/mL	< 0.001		0.067	0.001		0.947
GGT > 36 IU/mL	< 0.001			0.001		
Triglycerides, mg/dL	0.011		0.059	0.018		0.957
Triglycerides > 106 mg/dL	0.009			0.009		
LDL, mg/dL	0.651		-	0.180		-
HOMA index	0.038		0.073	0.278		0.105
HOMA index > 1.2	0.150			0.489		
HOMA index > 3	0.002			0.018		

Data are given as mean  $\pm$  SD or as *n* (%).

<sup>1</sup>Significant alcohol intake was defined as > 30 g per day for male and > 20 g per day for female. At the multivariate logistic regression model only patients with available data for all the variables were included. The cut-off for inclusion was a *P* value < 0.10 in the univariate model. AST: Aspartate transaminase; CAP: Control attenuation parameter; GGT: Gamma glutamyltransferase; CI: Confidence interval; OR: Odds ratio; HOMA: Homeostasis model assessment; LDL: Low-density lipoprotein.

Overall, patients with HBV infection presented higher rates of both carotid plaques (20.9% *vs* 18.4%) and subclinical atherosclerosis (24.4% *vs* 19.4%), though these differences did not reach statistical significance (*P* = 0.31 and *P* = 0.14, respectively). When the three groups were analyzed separately, taking into account the state of HBV infection, we learnt that patients with CHB had higher rates of carotid plaques (32.7%) and subclinical atherosclerosis (40.8%) than controls (18.4% and 19.4%, respectively), as shown in **Figure 1**. However, the rates were similar when only ICs and controls were compared (carotid plaques: 17.1% *vs* 18.4%, *P* = 0.446; subclinical atherosclerosis: 19.1% *vs* 19.4%, *P* = 0.525). Although the typical cardiovascular risk factors were linked with both carotid plaques and subclinical atherosclerosis on the univariate analysis, on the multivariate (**Table 2**) the only factors independently associated with the presence of subclinical atherosclerosis were older age (OR 1.45, *P* < 0.001), diagnosis of CHB (OR 1.23, *P* < 0.001) and diabetes (OR 1.13, *P* = 0.028). Similar results were observed



**Figure 1** Rate of carotid plaques and subclinical atherosclerosis (defined as intima-media thickness  $\geq 1.2$  mm and/or presence of atheroma plaques) in the overall cohort. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ .

regarding the carotid plaques, with age over 50 years (OR 1.43,  $P < 0.001$ ) and CHB (OR 1.18,  $P = 0.004$ ) as independent risk factors.

Though this is a prospective study, due to the different number of HBV-infected subjects included in each group, a propensity score analysis including all patients with CHB ( $n = 49$ ) and a cohort with the same number of IC and controls, balanced by age, sex and main cardiovascular risk factors, was carried out. The multivariate analysis of this propensity score revealed similar results as shown the analysis performed with the overall cohort, with older age (OR 1.30,  $P = 0.01$ ) and CHB state (OR 1.26,  $P = 0.03$ ) as independent risk factors associated with the presence of carotid plaques (Table 3).

### Carotid plaques and subclinical atherosclerosis in CHB and HBV ICs

Overall, 49 (24.4%) patients had subclinical atherosclerosis, including 42 (20.9%) with carotid plaques, 19 (9.5%) with increased IMT ( $\geq 1.2$  mm) and 12 (6%) with both findings. The prevalence of both subclinical atherosclerosis ( $P = 0.003$ ) and carotid plaques ( $P = 0.019$ ) was higher in patients with CHB than ICs (Figure 2). Liver cirrhosis was associated with an increased risk of subclinical atherosclerosis (42.0% vs 23.0%) although the difference did not reach statistical significance ( $P = 0.13$ ). The impact of CHB on the presence of subclinical atherosclerosis remained when patients were stratified by age (Figure 2). In those  $\leq 50$  years, the prevalence of subclinical atherosclerosis was 12.5% in CHB patients and only 2.2% in ICs ( $P = 0.058$ ). In patients aged over 50 years, those with CHB also had a higher prevalence of subclinical atherosclerosis (68.0% vs 45.8%,  $P = 0.051$ ). Age was strongly associated with the presence of subclinical atherosclerosis ( $P < 0.001$ ). On multivariate analysis, factors independently associated with the presence of subclinical atherosclerosis were older age (OR 1.11,  $P < 0.001$ ), increased values of gamma-glutamyltransferase (OR 5.9,  $P = 0.007$ ) and CHB (OR 3.35,  $P = 0.017$ ). When age was introduced as a categorical variable (threshold of 50 years), both CHB and age remained as predictive factors of subclinical atherosclerosis (Table 4).

In terms of carotid plaques, impact of CHB was especially important in patients aged  $\leq 50$  years (Figure 2). On the multivariate analysis, only age (age  $> 50$  years, OR 1.45, 95% confidence interval 1.30-1.62,  $P < 0.001$ ) and increased gamma-glutamyltransferase levels (gamma-glutamyltransferase  $> 36$  IU/mL, OR 1.19, 95% confidence interval 1.04-1.37,  $P = 0.012$ ) independently impacted the presence of carotid plaques.

Four patients with CHB and liver cirrhosis presented a NAFLD score  $> 0.675$ , suggesting significant fibrosis. Two of them had subclinical atherosclerosis, but none had a history of diabetes, and their body mass index was  $< 25$  kg/m<sup>2</sup> and CAP  $< 250$  dB/m. Otherwise, they presented an HBV DNA  $> 2000$  IU/mL, suggesting that fibrosis was likely related to CHB.

**Table 3** Factors associated with the presence of carotid plaques in a propensity score matched by age, sex and main cardiovascular risk factors

	Groups of study			Univariate analysis	Multivariate analysis	
	Chronic hepatitis B, n = 49	Inactive carriers, n = 49	Controls, n = 49	P value	OR (95%CI)	P value
Age, yr	48.4 ± 12.0	48.7 ± 13.0	47.1 ± 11.3	0.78		
Age > 50 yr (%)	25 (51.0)	24 (49.0)	23 (46.9)	0.92	1.30 (1.12-1.50)	0.01
Male sex (%)	31 (64.6)	31 (63.3)	31 (63.3)	1		0.72
Caucasian race (%)	35 (72.9)	32 (65.3)	38 (77.6)	0.41		-
Cardiovascular risk factors (%)						
Tobacco exposure	15 (31.3)	13 (26.5)	16 (32.7)	0.80		0.71
Hypertension	11 (22.9)	10 (20.4)	11 (22.4)	0.96		0.40
Diabetes	4 (8.3)	5 (10.2)	3 (6.1)	0.76		0.57
Dyslipidemia	3 (6.3)	3 (6.1)	5 (10.2)	0.68		0.54
Central obesity	10 (20.8)	10 (20.4)	10 (20.4)	1		0.06
BMI, kg/m <sup>2</sup>	26.0 ± 3.9	26.3 ± 4.9	26.4 ± 3.8	0.90		0.16
ALT, IU/mL	48.6 ± 9.4	26.9 ± 9.4	25.2 ± 9.6	< 0.001		0.08
GGT, IU/mL	60.7 ± 87.9	42.7 ± 106.0	37.7 ± 50.9	0.45		0.07
LDL, mg/dL <sup>1</sup>	116.9 ± 30.7	120.3 ± 32.6	112.7 ± 35.3	0.72		0.87
Triglycerides, mg/dL	96.4 ± 46.3	99.0 ± 57.5	115.3 ± 77.1	0.30		0.41
HOMA index <sup>2</sup>	4.2 ± 3.5	4.7 ± 5.8	2.6 ± 2.6	0.14		0.40
Transient elastography, kPa	11.3 ± 10.9	5.7 ± 3.0	5.0 ± 7.9	< 0.001		0.80
CAP, dB/m	227.4 ± 55.0	232.1 ± 48.6	251.5 ± 62.8	0.095		0.49
Chronic hepatitis B state (%)	49 (100)	0 (0)	0 (0)	< 0.001	1.26 (1.09-1.47)	0.03

<sup>1</sup>Only available for 34 controls.

<sup>2</sup>Only available for 25 non-infected controls. ALT: Alanine transaminase; CAP: Control attenuation parameter; GGT: Gamma glutamyltransferase; BMI: Body mass index; LDL: Low-density lipoprotein; CI: Confidence interval; OR: Odds ratio; HOMA: Homeostasis model assessment.

## DISCUSSION

The results of this prospective collaborative study including well-characterized HBeAg negative chronic HBV infection show that CHB is independently associated with the presence of both carotid plaques and subclinical atherosclerosis. These results suggest that HBV infection may have a role as a cardiovascular risk factor in naïve patients with CHB.

There are few studies assessing the potential effect of HBV infection on development of carotid atherosclerosis, and they are all cross-sectional with a limited number of HBsAg-positive populations (Supplementary Table 1). In two of these studies, an association was observed between HBsAg positivity and early atherosclerosis[11,20]. The severity of liver disease was not determined in any of these studies, and therefore no data on the possible impact of CHB was reported. In our cohort, similar to HCV and HIV, patients with HBV infection had greater risk of subclinical atherosclerosis and carotid plaques than controls. In this line, a study focusing on early atherosclerosis in liver disease (NAFLD, HCV and HBV) found that all three conditions were strongly associated with early atherosclerosis (OR 1.96, 1.61 and 1.40 respectively), regardless of the patients' classical risk factors, including insulin resistance and metabolic syndrome [20].



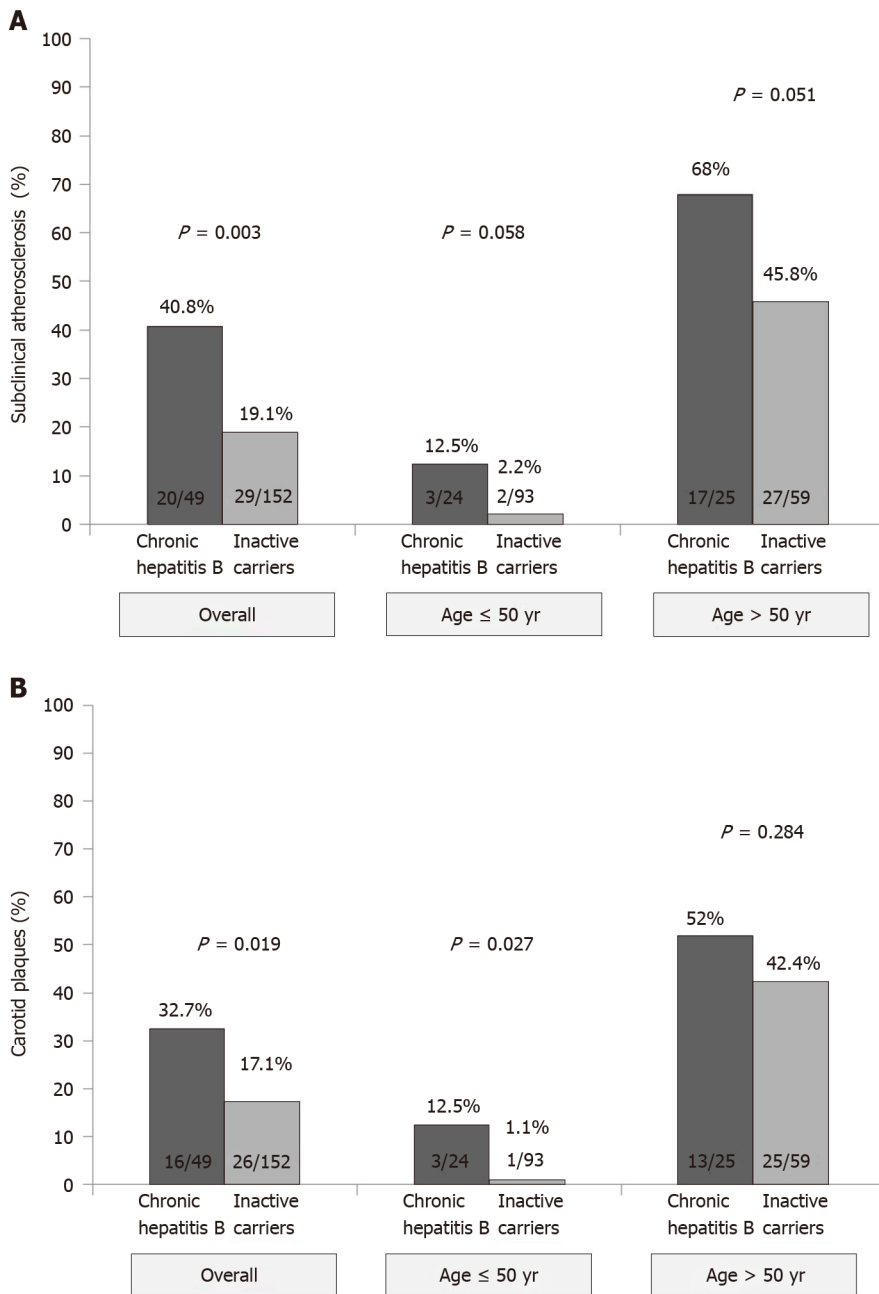
**Table 4** Baseline characteristics and analyses of factors associated with the presence of subclinical atherosclerosis of patients with hepatitis B virus infection

	Subclinical atherosclerosis	No subclinical atherosclerosis	Univariate analysis	Multivariate analysis		Adjusted multivariate analysis	
	n = 49	n = 152	P value	OR (95%CI)	P value	OR (95%CI)	P value
Age, yr	57 (53.5-62.0)	42 (33.0-52.0)	< 0.001	1.10 (1.06-1.16)	< 0.001	1.19 (1.12-1.25)	< 0.001
Age > 50 yr (%)	45 (91.8)	46 (30.3)	< 0.001	21.9 (6.7-71.8)	< 0.001		
Male sex (%)	31 (63.3)	84 (55.3)	0.207	-	-		
BMI, kg/m <sup>2</sup>	70.0 (64-77)	69.5 (62-80)	0.261	-	-		
Central obesity (%)	13 (26.5)	24 (16.0)	0.079	-	0.298		0.356
Tobacco exposure (%)	22 (44.9)	38 (25.2)	0.008	-	0.208		0.920
Alcohol intake <sup>1</sup> (%)	14 (28.5)	20 (13.5)	0.016	-	0.876		0.092
Arterial hypertension (%)	18 (36.7)	20 (13.2)	0.001	-	0.789		0.419
Diabetes mellitus (%)	5 (10.2)	5 (3.3)	0.067	-	0.994		0.457
Dyslipidemia (%)	16 (32.6)	18 (11.9)	0.001	-	0.876		0.786
Chronic hepatitis B (%)	20 (40.8)	28 (18.4)	0.002	3.35 (1.20-9.10)	0.017	1.89 (1.75-2.04)	< 0.001
Liver cirrhosis (%)	5 (10.2)	7 (4.6)	0.138	-	-		
ALT, IU/mL	29 (22.0-43.5)	24 (17.0-34.0)	0.310	-	--		
ALT > ULN (%)	10 (20.4)	22 (14.5)	0.220	-	-		
GGT, IU/mL	31 (18.0-62.5)	20 (16.0-28.0)	< 0.001	-	0.120		
GGT > ULN (%)	16 (32.7)	8 (5.3)	< 0.001	5.90 (1.60-21.40)	0.007	1.27 (1.19-1.36)	< 0.001
HbA1c, %	5.5 (5.3-5.8)	5.4 (5.1-5.6)	0.004	-	0.78		0.551
HbA1c ≥ 6% (%)	7 (14.6)	6 (4.1)	0.018	-	-		
HOMA index	3.3 (2.2-6.0)	2.4 (1.7-3.6)	0.038	-	-		0.054
HOMA index > 3 (%)	22 (45.8)	51 (34.2)	0.102	-	-		
HBsAg, logIU/mL	3.2 (2.5-3.6)	3.3 (2.4-4.0)	0.321	-	-		
HBV DNA, logIU/mL	3.1(2.4-3.8)	2.9 (2.3-3.5)	0.533	-	-		
Transient elastography, kPa	6.2(4.2-10.3)	5.2 (4.2-6.9)	0.059	--	0.327	1.01 (1.00-1.01)	< 0.001
CAP, dB/m	246 (210.0-289.0)	213 (185.0-261.5)	0.004	--	0.220	1.000 (1.000-1.001)	0.006
CAP > 227 dB/m (%)	29 (67.4)	51 (38.3)	0.001	--	0.172		

Data are given as mean ± SD or as n (%).

<sup>1</sup>Significant alcohol intake was defined as > 30 g per day for male and > 20 g per day for female. At the multivariate logistic regression model only patients with available data for all the variables were included (n = 250). The cut-off for inclusion was a P value < 0.10 in the univariate model. ALT: Alanine transaminase; BMI: Body mass index; HBV: Hepatitis B virus; CI: Confidence interval; CAP: control attenuation parameter; HbA1c: Hemoglobin A1c; GGT: Gamma glutamyltransferase; OR: Odds ratio; ULN: Upper limit of normality; HOMA: Homeostasis model assessment; HBsAg: Hepatitis B surface antigen.

The suggested mechanisms to explain HBV-related atherosclerosis is direct vascular damage by the virus and particularly accelerated oxidative damage and the pro-inflammatory state of chronic HBsAg carriers[21]. Knowledge about the immune response in HBV-infected patients has increased considerably in recent years[9]. The production of proinflammatory cytokines (*e.g.*, interleukin1b, tumor necrosis factoralpha) steadily increases during early life until it reaches the state of chronic low-



**Figure 2** Rate and impact of age and hepatitis B e antigen negative phase of infection (chronic hepatitis vs inactive carriers) in subclinical atherosclerosis and carotid plaques in the cohort of patients chronically infected by hepatitis B virus. A: Subclinical atherosclerosis; B: Carotid plaques.

grade systemic inflammation that occurs in elderly persons[22]. HBeAg-negative CHB [3] has been linked with a propensity to mount proinflammatory immune reactions [9]. In this population, liver inflammation is triggered by HBV-specific CD8 T cells, and it is associated with increased levels of chemokines and natural killer cell activation[23]. This proinflammatory state is independent of ALT levels and even HBV DNA levels, which usually fluctuate in this stage of the disease[24]. However, it has been clearly associated with progression of liver disease[9].

In our study, neither ALT levels nor HBV DNA were associated with an increased prevalence of subclinical atherosclerosis. This fact may be explained by the inclusion of patients with CHB with normal ALT but increased values of HBV DNA and liver damage at liver biopsy. On the other hand, some of the patients with liver cirrhosis presented relatively low HBV DNA levels. Older age and CHB status were independent factors associated with increased carotid plaques and subclinical atherosclerosis, in line with the proinflammatory state induced by older age and progression of liver damage.

Serum paraoxonase-1 and arylesterase activities, plasma free sulfhydryl groups and total antioxidant capacity, all factors associated with increased susceptibility to atherogenesis[24,25], are lower in HBV patients than in non-infected controls[26]. This finding can also contribute to the development of atherosclerosis in patients with HBV infection. Moreover, the association between fibrosis progression and exacerbated immune responses in patients with CHB is well established[9,10,27], so this dysfunctional immunological response might also bring an increase in cardiovascular risk.

Accordingly, HCV infection has been linked with increased prevalence of carotid plaques in those patients with evidence of advanced liver fibrosis[4]. In that study, Petta *et al*[4] showed that 73 of 174 HCV patients (42%) had carotid plaques, with older age and liver fibrosis as independent factors associated with carotid atherosclerosis, results in line with our findings because age and CHB were the two variables independently linked with increased risk of both carotid plaques and subclinical atherosclerosis. The role of liver damage is especially relevant in view of the lack of statistical differences when HBV ICs were compared with controls, suggesting that HBV infection may predispose to cardiovascular risk only when it is associated with a proinflammatory state, as described in patients with CHB[9,27].

This study has some limitations. First, the fact that only naïve patients were included turned out in a relatively low number of patients with HBeAg negative CHB and inferior to the cohort of HBV ICs. However, these patients were well characterized, and all met the European Association for the Study of the Liver criteria for CHB, including 24% with liver cirrhosis. Second, there were some differences among the groups. In order to minimize this potential bias, a propensity score was carried out, confirming the role of CHB status as cardiovascular risk factor. Moreover, data presented herein derived from a prospective, collaborative cohort of well-characterized patients, including different ethnicity and therefore HBV genotypes.

Interestingly, since reversion of liver fibrosis in patients with CHB is possible due to nucleos(t)ide analog therapy[28], it would be appealing to assess the potential impact of oral antiviral therapy on early atherosclerosis related to HBV infection, especially to view the effect of antiviral treatment for HCV in the overall cardiovascular risk and specifically in the carotid plaques[7].

## CONCLUSION

In conclusion, in this prospective, case-control collaborative study, presence of subclinical atherosclerosis and carotid plaques were more frequent in patients with HBV infection than controls. The presence of liver damage was an independent factor associated with subclinical atherosclerosis and carotid plaques, regardless of the classical cardiovascular factors.

## ARTICLE HIGHLIGHTS

### **Research background**

There is an increased risk of atherosclerosis in patients with chronic hepatitis C and also in individuals with human immunodeficiency virus infection.

### **Research motivation**

There is scarce data on the potential role of hepatitis B virus infection as a cardiovascular risk factor.

### **Research objectives**

To assess whether the stage of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B virus infection impacts the presence of both carotid plaques and subclinical atherosclerosis and to evaluate if the risk of both carotid plaques and subclinical atherosclerosis in HBeAg-negative patients differ to those of healthy controls.

### **Research methods**

Prospective case-control study with 402 subjects prospectively recruited at the outpatient clinic. Anthropomorphic and metabolic measures, liver stiffness and carotid Doppler ultrasound were performed.

**Research results**

Patients with HBeAg-negative chronic hepatitis B presented a higher rate of carotid plaques than healthy controls (32.7% *vs* 18.4%,  $P = 0.002$ ), but no differences were observed between controls and hepatitis B inactive carriers. HBeAg-negative chronic hepatitis B was an independent risk factor for carotid plaques as well as age, dyslipidemia and central obesity.

**Research conclusions**

These results suggest that hepatitis B infection may have a role as a cardiovascular risk factor in patients with chronic hepatitis B.

**Research perspectives**

Further studies should assess the potential impact of oral antiviral therapy on early atherosclerosis related to hepatitis B virus infection.

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