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CLINICAL STUDIES

Hepatic steatosis and insulin resistance are associated with severe fibrosis in patients with chronic hepatitis caused by HBV or HCV infection

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Keywords
HBV – HCV – steatosis

Abbreviations
CHB, chronic hepatitis B; HBV, hepatitis B virus; HBV-DNA, hepatitis B virus deoxyribonucleic acid; HOMA, homeostasis model assessment; IR, insulin resistance.

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Abstract

Background and aims: Steatosis and insulin resistance (IR) are the major disease modifying in patients with chronic hepatitis C (CHC). Only few studies evaluated these features in patients with chronic hepatitis B (CHB). We aimed to assess the prevalence and the factors related to steatosis and IR in CHB patients, compared with CHC subjects, and to evaluate the potential association between these features and fibrosis severity. *Material and methods:* One hundred and seventy consecutive patients with CHB (28 HBeAg positive, 142 HBeAg negative), were evaluated using liver biopsy and metabolic measurements and matched for sex, age and body mass index with 170 genotype 1 CHC patients. IR was defined if HOMA-IR > 2.7. All biopsies were scored for grading and staging by Scheuer's score, and the steatosis was considered significant if $\geq 10\%$. *Results:* The prevalence of significant steatosis was similar in both CHB and CHC patients (31 vs. 38%; $P = 0.14$). IR rate was significantly higher in CHC than in CHB patients (42 vs. 26%; $P = 0.002$). Severe fibrosis (F3–F4), at multivariate analysis, was independently associated with older age (OR 1.050, 95% CI 1.009–1.093), steatosis $> 10\%$ (OR 4.375, 95% CI 1.749–10.943), and moderate–severe necroinflammatory activity (OR 8.187, 95% CI 2.103–31.875), regardless of HBeAg status, in CHB patients, and with older age (OR 1.080, 95% CI 1.028–1.136), IR (OR 2.640, 95% CI 1.110–6.281), steatosis $> 10\%$ (OR 3.375, 95% CI 1.394–8.171), and moderate–severe necroinflammatory activity (OR 8.988, 95% CI 1.853–43.593) in CHC patients. *Conclusions:* CHB patients had high steatosis prevalence, similar to CHC controls, but lower IR rate. Both steatosis and IR in CHC, and only steatosis in CHB, are independently associated with fibrosis severity.

Steatosis and insulin resistance (IR) are the key findings in patients with nonalcoholic fatty liver disease (NAFLD) (1). Similarly, in patients with chronic hepatitis C (CHC), where HCV seems able to directly interfere with mechanisms leading to steatosis and IR (2, 3), these features represent two frequent findings able to negatively influence the severity of liver disease (4–6) and the likelihood of response to antiviral therapy (4, 7). Instead only few studies evaluated the prevalence and the role of both steatosis and IR in patients with chronic hepatitis B (CHB).

Some studies, performed in population different for ethnicity, demographical, clinical and metabolic factors, reported a prevalence of steatosis in CHB patients ranging from 4.5 to 71%, observing that fat accumulation was independently associated with metabolic factors, such as high body mass index (BMI), dislipidaemia, IR and diabetes (8–32). In addition, these studies did not find any association between steatosis and viral factors, and did not identify steatosis as an independent risk factor for severity of fibrosis in CHB patients.

Contrasting results reported similar or lower IR rate in HBV patients compared with HCV subjects (14, 29–31, 33, 34), and similar or higher IR rate in HBV patients compared with healthy controls (26, 28, 35, 36), even if no data showed an association between viral characteristics of HBV and IR. Interestingly only two of these studies identified IR (32) and the metabolic syndrome (37), the clinical expression of IR, as significant risk factors for cirrhosis.

Therefore, the aims of this study were to assess the prevalence and the factors associated with steatosis and IR in CHB patients, compared with matched CHC subjects, and to evaluate the potential association between these features and severity of fibrosis.

Materials and methods

Patients

This study was carried out on a sample of 170 consecutive patients with CHB, recruited at the GI & Liver Unit of the University Hospital in Palermo, between January 2000 and December 2008. Patients were included if they had abnormal alanine aminotransferase (ALT) from at least 6 months, HBV-DNA > 2000 IU/ml, and a histological diagnosis of CHB with any degree of fibrosis, including cirrhosis. As control group we selected a cohort of 170 G1 CHC patients matched for sex, age and BMI, observed in the same period of time, and characterized by the presence of anti-HCV and HCV-RNA, and with a liver histology of chronic hepatitis (any degree of fibrosis, including cirrhosis).

Exclusion criteria were: (i) advanced cirrhosis (Child–Pugh B and C); (ii) hepatocellular carcinoma; (iii) other causes of liver disease or mixed aetiologies; (iv) alcohol consumption > 20 g/die in the last 6 months below the biopsy, evaluated by patient history; (v) HIV infection; (vi) previous treatment with antiviral therapy in the last years before liver biopsy; previous treatment with immunosuppressive drug and/or regular use of steatosis-inducing drugs; (vii) previous diagnosis of type 1 diabetes mellitus; (viii) active IV drug addiction.

This study was performed in accordance with the principles of the Declaration of Helsinki, and its appendices, and with local and national laws. Approval was obtained from the hospital's Internal Review Board and the Ethical Committee, and written informed consent from all patients and controls.

Clinical and laboratory assessment

Clinical and anthropometric data were collected at the time of liver biopsy. BMI was calculated on the basis of weight in kilograms and height (in metres). and subjects were classified as normal weight (BMI, 18.5–24.9 kg/m^2), overweight (BMI, 25–29.9) and obese (BMI > 30).

The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association using a value of fasting blood glucose of ≥ 126 mg/dl on at least two occasions (38). In patients with a previous diagnosis of type 2 diabetes, current therapy with oral hypoglycaemic agents was documented.

A 12-h overnight fasting blood sample was drawn at the time of biopsy to determine serum levels of ALT, total cholesterol, triglycerides and plasma glucose concentration. Serum insulin was determined using a two-site enzyme ELISA (Mercodia Insulin ELISA, Arnika). IR was determined by the homeostasis model assessment (HOMA) method, using the following equation (39): Insulin resistance (HOMA-IR) = Fasting insulin (μU/ml) × Fasting glucose (mmol/L)/22.5. HOMA-IR has been validated in comparison with the euglycaemic/hyperinsulinaemic clamp technique in both diabetic and nondiabetic subjects (40). HOMA-IR values > 2.7 were considered to indicate IR; this cut-off corresponds to the upper quartile of a previously published control Italian population (41).

Hepatitis B virus-infected patients were tested at the time of biopsy for HBs, HBeAg, anti-HBe and anti-HDV IgG, using commercial enzyme immunoassays (Dia Sorin, Saluggia, Italy). HBV-DNA was quantified by bDNA (Versant HBV 3.0, Siemens Medical Solutions Diagnostics Europe, Dublin, Ireland; range 357–17 857 000 IU/ml). HCV-infected individuals were tested at the time of biopsy for HCV-RNA using qualitative PCR (Cobas Amplicor HCV Test version 2.0; limit of detection: 50 IU/ml). HCV-RNA-positive samples were quantified by Versant HCV-RNA 3.0 bDNA (Bayer Co. Tarrytown, NY, USA) expressed in IU/ml. Genotyping was performed by INNO-LiPA (HCV II, Bayer HealthCare, Berkeley, CA, USA).

Assessment of histology

Slides were coded and read by a single pathologist (D. C.) unaware of the patient's identity and history. A minimum length of 15 mm of biopsy specimen or the presence of at least 10 complete portal tracts was required (42). Biopsies were classified according to the Scheuer numerical scoring system (43).

The percentage of hepatocytes containing fat was determined for each × 10 field. An average percentage of steatosis was then determined for the entire specimen. Steatosis was assessed as the percentage of hepatocytes containing fat droplets (minimum, 5%), and evaluated as continuous variable. Steatosis was also classified as: mild > 5 to < 10%, moderate/severe ≥ 10%.

Statistics

Continuous variables were summarized as mean ± SD and categorical variables as frequency and percentage. Multiple logistic regression models were used to assess the relationship of fibrosis, steatosis, necroinflamatory activity and IR to demographical, virological, metabolic and histological characteristics of patients, in both CHB and CHC patients. In the first model, the dependent variable was fibrosis coded as 0 = F1–F2; or 1 = F3–F4. As candidate risk factors for severe fibrosis (F3–F4) we selected age, gender, BMI, baseline ALT, total cholesterol, triglycerides, blood glucose, insulin, HOMA score, IR, diabetes, HBV-DNA levels (for HBV patients), virological status (for HBV patients), HCV-RNA levels (for HCV patients), steatosis > 10%, and moderate–severe necroinflammatory activity. In the second model, the dependent variable was steatosis coded as 0 ≤ 10%; or 1 ≥ 10%. In the third model, the dependent variable was

necroinflammatory activity coded as 0 = mild; or 1 = moderate–severe. In the fourth model, the dependent variable was IR coded as 0 = HOMA $\leq$ 2.7; or 1 = HOMA $>$ 2.7.

Variables found to be associated with the dependent variable at univariate analyses (probability threshold, $P \leq 0.10$) were included in multivariate regression models. To avoid the effect of the co-linearity, diabetes, IR, HOMA-score, blood glucose levels and insulin levels, were not included in the same multivariate model. Similarly we did not include necroinflammatory activity and ALT levels in the same multivariate model. Regression analyses were performed using PROC LOGISTIC, PROC REG and subroutine in SAS (SAS Institute Inc., Cary, NC, USA) (44).

Results

Patients' features and histology

Baseline features of HBV and HCV patients are shown in Table 1. Obesity was identified in about 10% of cases in both groups. IR rate was significantly higher in CHC than in CHB patients (42.2 vs. 25.9%; $P = 0.002$), as well as type 2 diabetes (8.8 vs. 3.6%; $P = 0.04$). At liver biopsy the prevalence of steatosis was similar in both CHB and CHC patients (40 vs. 47%), and a moderate–severe grade was identified in approximately 30–40% of the cases in both the groups. In particular, steatosis $\geq$30% was found in 11.2% of HBV (19/170), compared with 14.7% (25/170) of HCV patients ($P = 0.33$). Severe fibrosis was found in about a quarter of cases in both HCV- and HBV-infected patients.

Table 1. Demographical, laboratory, metabolic, virological and histological features of 340 patients with chronic hepatitis B and genotype 1 chronic hepatitis C

Variable	Chronic hepatitis B ($n = 170$)	Chronic hepatitis C ($n = 170$)	P
Mean age (years)	40.2 ± 14.0	42.5 ± 10.3	0.11
Gender			
Male/female	121 (71.2)/49 (28.8)	121 (71.2)/49 (28.8)	1.00
Virological status			
HBeAg positive	28 (16.5)		
Anti-HBe positive*	142 (83.5)	–	–
Log_{10}HBV-DNA	4.9 ± 1.5	–	–
Log_{10}HCV-RNA	–	5.5 ± 0.6	–
Mean body mass index (kg/m^2)	25.5 ± 3.5	25.8 ± 3.6	0.54
Body mass index (kg/m^2)			
$<$ 25	70 (41.2)	65 (38.2)	
25–29.9	79 (46.5)	88 (51.8)	
$\geq$30	21 (12.3)	17 (10.0)	
Type 2 diabetes			
Absent/present	164 (96.4)/6 (3.6)	155 (91.2)/15 (8.8)	0.04
Alanine aminotransferase (IU)	102.9 ± 97.3	92.6 ± 74.8	0.27
Cholesterol (mg/dl)	190.5 ± 44.4	171.1 ± 37.8	$<$ 0.001
Triglycerides (mg/dl)	101.0 ± 53.8	106.2 ± 70.3	0.49
Blood glucose (mg/dl)	90.5 ± 20.1	90.2 ± 23.0	0.80
Insulin (μU/ml)	10.4 ± 7.1	12.3 ± 8.1	0.02
HOMA score	2.38 ± 1.81	2.78 ± 1.80	0.04
Insulin resistance (HOMA $>$ 2.7)			
Absent/present	126 (74.1)/44 (25.9)	100 (58.8)/70 (42.2)	0.002
Histology at biopsy			
Steatosis			
Absent ($<$ 5%)	102 (60.0)	90 (52.9)	
Present ($\geq$5%)	68 (40)	80 (47.1)	0.19
Absent/mild ($<$ 10%)	117 (68.8)	104 (61.2)	
Moderate/severe ($\geq$10%)	53 (31.2)	66 (38.8)	0.14
Stage of fibrosis			
1	65 (38.2)	55 (32.4)	
2	61 (35.8)	75 (44.1)	
3	26 (15.3)	30 (17.6)	
4	18 (10.7)	10 (5.9)	0.95
Grade of activity			
1	53 (31.2)	42 (24.7)	
2	64 (37.6)	100 (58.8)	
3	53 (31.2)	28 (16.5)	0.25

*14 patients were anti-HDV positive.

HOMA, homeostasis model assessment; HCV-RNA, hepatitis C virus ribonucleic acid; HBV-DNA, hepatitis B virus deoxyribonucleic acid.

Table 2. Univariate and multivariate analysis of risk factors associated with moderate–severe steatosis (≥10%) in 340 patients with chronic hepatitis B and genotype 1 chronic hepatitis C

Variable	Steatosis < 10%	Steatosis ≥ 10%	Univariate analysis *P* value	Multivariate analysis OR (95% CI)	*P* value
Chronic hepatitis B ($n = 170$)					
	$n = 117$	$n = 53$			
Age (years)	37.4 ± 13.9	46.6 ± 12.1	< 0.001	1.043 (1.011–1.076)	0.009
Gender					
Male vs. female	77/40	44/9	0.02	0.298 (0.111–0.805)	0.01
Virological status					
HBeAg/anti-HBe*	24/93	4/49	< 0.001	0.710 (0.198–2.551)	0.60
Body mass index (kg/m^2)	24.9 ± 3.1	26.9 ± 3.2	< 0.001	1.136 (1.005–1.284)	0.04
Insulin resistance (HOMA > 2.7)					
Absent/present	98/19	28/25	< 0.001	3.815 (1.700–8.560)	0.001
Genotype 1 chronic hepatitis C ($n = 170$)					
	$n = 104$	$n = 66$			
Age (years)	41.3 ± 11.0	44.4 ± 8.9	0.05	1.019 (0.985–1.054)	0.28
Body mass index (kg/m^2)	25.1 ± 3.5	26.9 ± 3.5	< 0.001	1.146 (1.034–1.271)	0.009
Alanine aminotransferase (IU)	86.4 ± 79.0	112.7 ± 95.6	0.05	1.003 (0.999–1.007)	0.16
Insulin resistance (HOMA > 2.7)					
Absent/present	73/31	27/39	< 0.001	2.761 (1.386–5.500)	0.004

*14 patients were anti-HDV positive.
HOMA, homeostasis model assessment.

Among HBV patients 28 (16.5%) were HBeAg positive, and 142 (83.5%) anti-HBeAg positive (14 of them anti-HDV positive). HBeAg- compared with anti-HBe-positive patients were younger ($P < 0.001$), had higher HBV-DNA viral load ($P < 0.001$) and ALT levels ($P < 0.001$) and had a lower prevalence of significant steatosis ($P = 0.03$) and severe fibrosis ($P = 0.01$).

Factors associated with moderate–severe steatosis

The univariate and multivariate comparison of variables between patients with and without moderate–severe steatosis (≥10%) is reported in Table 2, for both HBV and HCV patients. Multivariate logistic regression analysis (Table 2, top) showed that the following features were independently linked to moderate–severe steatosis (≥10%) in HBV patients: older age (OR 1.043, 95% CI 1.011–1.076, $P = 0.009$), male gender (OR 0.298, 95% CI 0.111–0.805, $P = 0.01$), high BMI (OR 1.136, 95% CI 1.005–1.284, $P = 0.04$) and IR (OR 3.815, 95% CI 1.700–8.560, $P = 0.001$). After the exclusion of 28 HBeAg-positive patients we obtained results similar to the exclusion of 14 anti-HDV-positive patients (data not shown).

In patients with CHC, we confirmed high BMI (OR 1.146, 95% CI 1.034–1.271, $P = 0.009$), and IR (OR 2.761, 95% CI 1.386–5.500, $P = 0.004$) as the only variables independently linked to moderate–severe steatosis (Table 2, bottom).

Factors associated with IR

Multivariate logistic regression analysis showed that the following features were independently linked to IR (HOMA > 2.7) in CHB patients: high BMI (OR 1.167, 95% CI 1.028–1.324, $P = 0.01$), and moderate–severe steatosis (OR 2.705, 95% CI 1.169–6.257, $P = 0.02$). After the exclusion of 28 HBeAg-positive patients we obtained results similar to the exclusion of 14 anti-HDV-positive patients (data not shown).

In patients with CHC, we found that high triglycerides levels (OR 1.014, 95% CI 1.005–1.022, $P = 0.002$), moderate–severe steatosis (OR 2.596, 95% CI 1.236–5.454, $P = 0.01$) and severe fibrosis (OR 2.571, 95% CI 1.061–6.228, $P = 0.03$) were independently associated with IR.

Considering the higher prevalence of IR observed in CHC patients compared with CHB subjects we performed a logistic model for IR including together all HBV and HCV patients, and evaluating aetiology as an additional dependent variable. Univariate and multivariate analysis (Table 3) showed that IR was independently associated with high BMI (OR 1.108, 95% CI 1.023–1.201, $P = 0.01$), high triglycerides levels (OR 1.006, 95% CI 1.001–1.011, $P = 0.02$), moderate–severe steatosis (OR 2.431, 95% CI 1.420–4.164, $P = 0.001$), severe fibrosis (OR 2.636, 95% CI 1.415–4.912, $P = 0.002$) and HCV infection (OR 2.032, 95% CI 1.209–3.414, $P = 0.007$).

Table 3. Univariate and multivariate analysis of risk factors associated with insulin resistance in 340 patients with chronic hepatitis B and genotype 1 chronic hepatitis C

Variable	No insulin resistance (HOMA $\leq$ 2.7) $n = 226$	Insulin resistance (HOMA $>$ 2.7) $n = 114$	Univariate analysis P value	Multivariate analysis OR (95% CI)	P value
Age (years)	39.4 $\pm$ 12.4	45.3 $\pm$ 11.3	$<$ 0.001	1.016 (0.992–1.040)	0.19
Gender					
Male vs. female	159/67	83/31	0.63	–	
Body mass index (kg/m^2)	25.1 $\pm$ 3.1	26.8 $\pm$ 3.7	$<$ 0.001	1.108 (1.023–1.201)	0.01
Alanine aminotransferase (IU)	100.3 $\pm$ 100.9	98.6 $\pm$ 71.6	0.87	–	
Cholesterol (mg/dl)	180.4 $\pm$ 41.3	176.0 $\pm$ 42.1	0.39	–	
Triglycerides (mg/dl)	96.5 $\pm$ 42.8	117.5 $\pm$ 78.4	0.002	1.006 (1.001–1.011)	0.02
Aetiology					
HCV vs. HBV	100/126	70/44	0.002	2.032 (1.209–3.414)	0.007
Histology at biopsy					
Steatosis					
$<$ 10% vs. $\geq$ 10%	171/55	50/64	$<$ 0.001	2.431 (1.420–4.164)	0.001
Fibrosis					
1–2 vs. 3–4	191/35	65/49	$<$ 0.001	2.636 (1.415–4.912)	0.002
Grade of inflammation					
1 vs. 2–3	75/151	21/93	0.004	1.432 (0.764–2.685)	0.26

HCV, hepatitis C virus; HBV, hepatitis B virus.

Factors associated with moderate–severe necroinflammatory activity

Multivariate logistic regression analysis showed that older age (OR 1.078, 95% CI 1.039–1.118, $P < 0.001$), high Log_{10} HBV-DNA (OR 1.705, 95% CI 1.187–2.451, $P = 0.004$) and higher ALT levels (OR 1.025, 95% CI 1.012–1.038, $P < 0.001$) were independently linked to moderate–severe necroinflammatory activity in CHB patients. After the exclusion of 28 HBeAg-positive patients we obtained results similar to the exclusion of 14 anti-HDV-positive patients (data not shown).

In patients with CHC, by multivariate analysis we confirmed high ALT levels (OR 1.020, 95% CI 1.008–1.032, $P = 0.001$) as the only variables independently linked to moderate–severe necroinflammatory activity.

Factors associated with severe fibrosis

The univariate and multivariate comparison of variables between patients with and without severe fibrosis (Scheuer score ≥ 3) is reported in Table 4 for both HBV and HCV patients. Multivariate logistic regression analysis (Table 4, top) showed that the following features were independently linked to severe fibrosis in HBV patients: older age (OR 1.050, 95% CI 1.009–1.093, $P = 0.01$), steatosis $> 10\%$ (OR 4.375, 95% CI 1.749–10.943, $P = 0.002$) and moderate–severe necroinflammatory activity (OR 8.187, 95% CI 2.103–31.875, $P = 0.002$). After the exclusion of 28 HBeAg-positive patients we obtained results similar to the exclusion of 14 anti-HDV-positive patients (data not shown).

In patients with CHC, older age (OR 1.080, 95% CI 1.028–1.136, $P = 0.002$), IR (OR 2.640, 95% CI 1.110–6.281, $P = 0.02$), steatosis $> 10\%$ (OR 3.375, 95% CI 1.394–8.171, $P = 0.007$) and moderate–severe necroinflammatory activity (OR 8.988, 95% CI 1.853–43.593, $P = 0.006$) were independently associated with severe fibrosis by multivariate logistic regression analysis (Table 4, bottom).

Discussion

In our study, we found a high prevalence (about 40%) of histological steatosis in HBV patients, similar to that observed in a matched control group of HCV-infected subjects. Our data agree with different clinical studies on the high prevalence of steatosis in HCV infection (4, 5, 34, 45, 46). In contrast, literature data (8–30) reported a high range (4.5–71%) in steatosis prevalence in HBV patients, probably because of the differences in demographical, clinical, biochemical, anthropometrical and metabolic characteristics of studied populations. However, it is noteworthy to emphasize that we found this high prevalence in a homogeneous cohort of HBV and HCV patients at low risk for steatosis, because nondrinkers, with a mean age of 40 years, and with a low rate of obesity, even if largely overweight.

According to different clinical evidences in HCV (5, 45, 46), and to two recent studies on HBV patients (14, 22), we identified in higher BMI and in IR, the most important factors independently associated with steatosis in both the two groups, confirming that metabolic factors are very relevant in the pathogenesis of steatosis in both these settings of patients. However, nevertheless the lack of association between viral load and steatosis probably because of the fluctuating levels of viremia in HBV and HCV infection, the higher observed steatosis prevalence in both HCV and HBV patients compared with those of NAFLD in the general population (47),

Table 4. Univariate and multivariate analysis of risk factors associated with severe fibrosis (F3–F4) in 340 patients with chronic hepatitis B and with genotype 1 chronic hepatitis C

Variable	Mild–moderate fibrosis (Scheuer score 1–2)	Severe fibrosis (Scheuer score 3–4)	Univariate analysis *P* value	Multivariate analysis OR (95% CI)	*P* value
Chronic hepatitis B ($n = 170$)					
	$n = 126$	$n = 44$			
Age (years)	37.0 ± 13.6	49.4 ± 10.8	< 0.001	1.050 (1.009–1.093)	0.01
Virological status					
HBeAg/anti-HBe*	26/100	2/42	0.01	0.287 (0.050–1.641)	0.16
Insulin resistance (HOMA > 2.7)					
Absent/present	104/22	22/22	< 0.001	2.076 (0.821–5.245)	0.12
Histology at biopsy					
Steatosis					
$< 10\%$ vs. $\geq 10\%$	100/26	17/27	< 0.001	4.375 (1.749–10.943)	0.002
Grade of inflammation					
1 vs. 2–3	50/25	3/42	< 0.001	8.187 (2.103–31.875)	0.002
Genotype 1 chronic hepatitis C ($n = 170$)					
	$n = 130$	$n = 40$			
Age (years)	40.6 ± 10.2	48.8 ± 8.2	0.002	1.080 (1.028–1.136)	0.002
Gender					
Male vs. female	86/44	35/5	0.009	0.329 (0.103–1.045)	0.06
Insulin resistance (HOMA > 2.7)					
Absent/present	87/43	13/27	< 0.001	2.640 (1.110–6.281)	0.02
Histology at biopsy					
Steatosis					
$< 10\%$ vs. $\geq 10\%$	89/41	15/25	< 0.001	3.375 (1.394–8.171)	0.007
Grade of inflammation					
1 vs. 2–3	41/89	2/38	< 0.001	8.988 (1.853–43.593)	0.006

*14 patients were anti-HDV positive.

HOMA, homeostasis model assessment.

suggests a potential additive direct role of both HBV and HCV in steatosis induction. Different lines of evidences clearly demonstrated that HCV is able to promote liver fatty accumulation (2, 46) with some differences according to viral genotype. In fact in G1 CHC, hepatic fat content is associated with clinical risk factors for NAFLD (45), and it is not responsive to antiviral therapy (48). Conversely, in G3 CHC, steatosis is more prevalent and severe (49, 50), correlates with the level of HCV replication (51, 52), does not affect the severity of fibrosis (53), disappears in patients with sustained virological response to antivirals, and re-occurs after viral relapse (54).

Some recent experimental evidences (55–57) also suggested that HBV, via HBx, is able to directly induce steatosis via LXR, Srebp and PPAR γ activation.

In this study, we also found that CHB patients, compared with sex-, age- and BMI-matched CHC subjects, had a significant lower rate of diabetes and IR. Different lines of evidences already reported a lower prevalence of diabetes in CHB patients compared with CHC subjects (58). Instead contrasting results, in populations different for baseline demographical, anthropometric and histological characteristics, reported similar or lower IR rate in HBV patients compared with HCV subjects (14, 29, 30, 34). Interestingly, we not only confirmed the association between metabolic risk factors, such as high BMI and triglyceride levels, and IR in both CHB (26, 28, 36) and CHC patients (5), but also identified in HCV infection an independent risk factors for IR presence. These data add further evidences on a direct role of HCV in induction of IR via interference with insulin signalling (2), and confirms recent clinical data in Asiatic populations (26, 28) that did not observe any relation between HBV infection and IR. However further experimental and clinical data are needed to definitively rule out the potential interference of HBV with insulin signaling.

In patients with CHB and CHC, several host and viral factors have been associated with the rate of fibrosis progression, which finally decides the prognosis of patients, leading over the years to the ultimate development of cirrhosis. In our study, we observed that liver necroinflammatory activity and metabolic factors were linked to fibrosis in both CHC and CHB. In particular, in line with literature data, in CHB we highlighted the important role of viral load to induce liver necroinflammation leading to fibrosis (59). This direct association between liver damage and viral load was not observed among CHC

patients, where only one retrospective analysis of six trials on G1 CHC patients underwent antiviral therapy, identified in liver inflammation a factor associated with HCV-RNA levels (60). Regarding the role of metabolic factors in promotion of liver fibrosis we confirmed the independent association of both IR and steatosis with fibrosis severity among CHC patients (4, 5). Similarly, among CHB patients, to our knowledge, this is the first study to provide evidence of an independent association between steatosis and fibrosis severity. Contrasting results obtained in other works investigating the potential association between fibrosis severity and steatosis in CHB (13–17, 19–22, 24, 25, 27, 28) may be explained by differences in the geographical origin and demographical characteristics of the patients, in the baseline severity of liver disease, and in the number of alcohol abusers. There was also a considerable heterogeneity in the methods for quantifying steatosis on liver biopsy, as well as in the cut-offs of steatosis used for statistical analyses among the different studies evaluating the impact of steatosis. However, our results about the association between steatosis and fibrosis severity in HBV patients, agree with growing evidences, in other clinical setting such as CHC and NAFLD, about the relevant role of liver fat accumulation in favouring disease progression (4, 53) via enhancement of oxidative stress that may induce both proinflammatory cytokines production and hepatic stellate cell activation, ultimately responsible of disease progression (61).

Instead, in our study, IR was not directly associated with severe fibrosis in CHB, as was observed in two recent studies on Asiatic population (31, 37), where patients were different for demographical, ethnical and virological characteristics. However, we do not rule out that IR could have an important role in liver disease progression, also in CHB, via inducing liver fat accumulation, as observed in our study.

The main limitation of this study, as well as of other cross-sectional studies, lies in its inability to analyse the temporal relationship among IR, steatosis and fibrosis. Lack of data on waist circumference, on presence of metabolic syndrome and on other potential confounders, such as increase in profibrogenic cytokines and adipocytokines, could also affect the interpretation of results. In addition, we cannot exclude the possibility that patients who falsely deny abuse of alcohol may be responsible for the observed prevalence of fibrosis and steatosis. A further methodological issue is the potential limit of external validity to new populations and settings. Our study included a cohort of nondrinking European patients, with a low prevalence of obesity, who were enrolled in a tertiary referral center for liver disease, limiting the broad application of the results.

In conclusion, this study shows that: (i) nondrinker patients with CHB had a high prevalence of steatosis, similar to that observed in matched CHC controls, and independently associated in both the two groups to high BMI and IR; (ii) CHB subjects were less insulin resistant than CHC individuals, considering HCV an additional risk factor for IR; (iii) both steatosis and IR in CHC, and only steatosis in CHB, especially together with hepatic necroinflammation, are independently associated with fibrosis severity. All these results therefore suggest the necessity to validate the potential role of insulin sensitizing approaches (behaviour therapy and or pharmacological treatment) together standard antiviral therapy, as instruments able to interfere with natural history of CHB and CHC.

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