

Effect of Alcohol Consumption on Serum Hepatitis C Virus RNA and Histological Lesions in Chronic Hepatitis C

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The role of alcohol intake in the occurrence of severe liver disease in chronic hepatitis C virus (HCV) carriers is still debated. A cross-sectional study has been conducted in 233 chronic hepatitis C virus carriers. Weekly self-reported alcohol consumption (SRAC) was evaluated, serum HCV RNA levels were measured by a branched DNA technique (Quantiplex 2.0) and HCV genotypes were determined. A liver biopsy was performed simultaneously and liver lesions were graded with the Knodell histological activity index. Data were examined by uni- and multivariate analyses. Alcohol consumption was relatively low (< 140 g/week in 193/233 patients [80%]). We found a highly significant correlation between SRAC and serum HCV RNA levels ($r = .26$, $P = .001$). Fibrosis was significantly correlated with age and alcohol consumption. These results suggest that in HCV carriers, alcohol consumption, even with low alcohol intake, increases viremia and hepatic fibrosis. Chronic HCV carriers should be advised to avoid regular alcohol intake. (HEPATOLOGY 1998;27:1717-1722.)

The natural course of HCV infection is probably modulated by several cofactors. The role of virus-related factors, namely genotypes and viremia has been extensively studied¹⁻⁷ but still remains debated.⁸ Up to now, immunological factors were underestimated but several arguments implicate the role of immune response in the course of the disease. Environmental factors and particularly alcohol intake are probably major factors,⁹⁻¹⁰ and the increased severity of the disease and the faster occurrence of cirrhosis in patients with HCV infection and heavy alcohol intake has been suggested in several studies.¹¹⁻¹² Moreover relations between alcohol and the immune response have been suggested with other viral infections.¹³ The aim of the present study is to describe the effect of moderate alcohol intake on serum HCV RNA levels and histological liver lesions in patients with chronic HCV infection.

PATIENTS AND METHODS

Patients

A cross-sectional study was conducted on a group of 233 patients to describe co-factors associated with outcome of HCV-related liver

disease. The 233 consecutive patients were admitted to the liver department from December 1994 to July 1996, for evaluation of chronic hepatitis caused by HCV. All patients were referred by a general practitioner to a senior physician of the department several months after the diagnosis of HCV infection and underwent transcutaneous liver biopsy, required for the diagnosis of chronic hepatitis caused by HCV. None of these patients was previously treated with interferon. We recorded simultaneously epidemiological data (age, sex, country of origin, date of discovery of HCV infection, and route of contamination) and chemical data (aspartate amino transferase and alanine amino transferase aminotransferases activities, prothrombin time, serum bilirubin, and gamma GT).

A questionnaire was proposed to every patient on the day of admission in the department and was conducted in every case by the same investigator in a face-to-face interview. The patients were not aware of the questionnaire before they were admitted in the department, but some of them were already told to stop drinking by the general practitioner before they were admitted to the liver department. This questionnaire was derived from the quantity-frequency format, based on the calculation of total weekly amounts, multiplying the daily intake by the number of drinking days. The interviewer was asked to record the regular weekday alcohol intake and extra consumption on the weekend. This questionnaire was based on the items of the "beverage specific quantity frequency questionnaire" validated in the 1988 US National Health Interview Survey¹⁴ and evaluated the self-reported alcohol consumption (SRAC). The retrospective questionnaire concerned successively two different periods, namely a typical week before the diagnosis of HCV chronic liver disease (and before any special recommendation concerning alcohol consumption); this was referred to as "past SRAC", and during the week before present hospitalization, this was referred to as "present SRAC."

Methods

The diagnosis of HCV infection was based on the detection of anti-HCV antibodies (Elisa Ortho Diagnosis System, Raritan, NJ). An analytical test was performed in every case to assess the profile of anti-HCV antibodies (RIBA Ortho). HCV genotype was determined with Inno Lipa (Innogenetics, Ghent, Belgium) in 197 patients in whom serum HCV RNA could be detected with polymerase chain reaction. The measurement of serum HCV RNA levels was performed in 233 cases with Quantiplex 2.0 branched DNA test (Chiron Corp., Emeryville, CA). When serum HCV RNA was undetectable with the Quantiplex test (36 cases), it was measured with polymerase chain reaction technique (Amplacor Roche Diagnostic System, Hoffman Laroche, Basel, Switzerland) which is able to detect lower amounts of HCV RNA.

Liver biopsy was performed in the 233 patients. Histological results were expressed with the Knodell histological activity index¹⁵; fibrosis and total activity score were quantified separately. The total activity index was the addition of the scores of periportal and bridging necrosis, intralobular and focal necrosis, and portal inflam-

Abbreviations: HCV, hepatitis C virus; SRAC, self-reported alcohol consumption.
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Received August 29, 1997; accepted February 4, 1998.
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0270-9139/98/2706-0035\$3.00/0

mation. All liver biopsies were examined by the same investigator, who was not aware of the epidemiological and virological data.

Statistical Analysis

Statistical analysis used the χ^2 test for percentage comparisons, the Student's *t* test for mean comparisons, and the Pearson correlation coefficient. Quantitative viremia and SRAC distributions were not normally distributed. Logarithmic transformations of these variables were used to run out statistical analysis. Knodell and activity scores were normally distributed and considered as quantitative variables. Multivariate analysis was done by using multiple regression analysis for quantitative dependent variables (viremia and activity score).

Fibrosis is an ordinal variable which can be considered as a four-level qualitative variable and/or a quantitative variable. In a first step, fibrosis score was considered as a qualitative variable. In univariate analysis, means of quantitative variables (age, SRAC, and serum HCV RNA) were compared by levels of fibrosis scores using ANOVA for unbalanced data¹⁶ in the framework of General Linear Model.¹⁷ Multivariate analysis was done by using polychotomous logistic regression.¹⁸ In a second step, fibrosis score was considered as a quantitative variable. Simple and multiple regression analyses¹⁹

were run out. The results were concordant and confirmed the findings of the first step. The SAS package software was used.

RESULTS

The description of the population and the epidemiological, virological, and histological characteristics are given in Table 1.

Alcohol Consumption The weekly SRAC was evaluated in grams of alcohol ingested per week. The weekly expression of alcohol intake was used because some patients have different drinking habits between weekdays and weekends. The following two variables were studied: alcohol consumption during the week preceding examination and SRAC concerning the period before the diagnosis of chronic liver disease caused by HCV.

Present SRAC. One hundred twenty three (52.8%) patients declared no alcohol consumption; 50 (21.5%) patients declared from 10 to 70 grams per week; 20 (8.6%) patients declared from 80 to 140 grams per week; 11 (4.7%) from 150 to 210 grams per week; and 29 patients (12.4%) declared > 210 grams per week.

TABLE 1. Clinical, Epidemiological, Biochemical, Virological, and Histological Data of the Cohort of 233 Patients

Variables	All		Drinkers at Inclusion		Non-drinkers at Inclusion		P
	n	%	n	%	n	%	
N	233		110	47.2	123	52.8	
Male gender	150	64.4	81	73.6	69	56.1	.005
Age							
20-29	32	13.7	16	14.6	16	13.0	.74
30-39	93	39.9	47	42.7	46	37.4	
40-49	54	23.2	25	22.7	29	23.6	
50-59	35	15.0	13	11.8	22	17.9	
60-72	19	8.2	9	8.2	10	8.1	
Caucasian origin	193	82.8	104	94.5	89	72.4	.001
Route of contamination							
drug addicts	96	41.2	55	50.0	41	33.3	.02
transfusion	62	26.6	22	20.0	40	32.5	
unknown	75	32.2	33	30.0	42	34.1	
Past SRAC:							
0 g/week	60	26.3	3	2.7	57	47.9	.001
10-350 g/week	129	56.6	78	71.6	51	42.9	
≥350 g/week	39	17.1	28	25.7	11	9.2	
median [min-max] g/week	11.4	[0-400]	24.3	[0-400]	2.8	[0-289]	
AST (\times upper limit of normal)*	1.2	[0.2-15]	1.15	[0.2-15]	1.2	[0.3-6.3]	
ALT (\times upper limit of normal)*	2.1	[0.2-21]	2.1	[0.2-13]	2.1	[0.3-13]	
GGT (\times upper limit of normal)*	1.2	[0.2-15]	1.3	[0.2-13]	1.06	[0.2-115]	
Prothrombin (%)*†	95	[65-118]	94	[65-111]	97	[68-118]	
Bilirubin (μ MOL/L)*†	11	[4-94]	10	[4-94]	11	[4-41]	
Knodell activity index: mean (sd)	5.4	(2.1)	5.4	(2.2)	5.4	(1.9)	.9
Knodell fibrosis index							
0	94	40.7	41	37.3	53	43.8	.36
1	100	43.3	54	49.1	46	38.0	
3	28	12.1	12	10.9	16	13.2	
4	9	3.9	3	2.7	6	5.0	
bDNA (meq \cdot 10 ⁻⁵ median) [min-max]	29	[2-541]	41	[2-541]	24	[2-243]	.003
Genotypes							
1a	38	16.3	18	16.4	20	16.3	.03
1b	78	33.5	31	28.2	47	38.2	
2a	16	6.9	5	4.6	11	8.9	
3a	40	17.2	25	22.7	15	12.2	
others	25	10.7	17	15.4	8	6.5	
undetermined	36	15.4	14	12.7	22	17.9	

*[min-max].

†median.

The mean SRAC was 90.1 (209 SD) grams per week in the whole group and 191 (273 SD) grams per week in the subgroup of patients who declared that they drank. The mean SRAC was 276 (410 SD) grams per week in men and 85 (136 SD) in women.

SRAC Before the Diagnosis. Alcohol consumption before the diagnosis of chronic hepatitis caused by HCV was evaluated with the same questionnaire. A mean period of 12.6 months (19 SD) separated the diagnosis of HCV infection and the hospitalization for liver biopsy. Sixty (25%) patients declared no alcohol consumption and 85 patients declared from 70 to 140 grams per week. Overall, one third of the drinkers stopped drinking even low amounts of alcohol when they became aware of the diagnosis of chronic HCV infection.

Present SRAC was inversely related to age ($r = -.15$, $P = .02$). It was lower in patients contaminated through blood transfusion (39 ± 93 g per week) than in patients contaminated through drug addiction (140 ± 250 g per week) ($P = .005$).

Genotyping Genotyping was performed in 197 patients. Genotyping was not possible in polymerase chain reaction-negative patients and in patients with a very weak polymerase chain reaction signal. Results are detailed in Table 1.

Relation Between Serum HCV RNA and Alcohol Consumption

Measurement of HCV RNA levels with branched DNA was performed in the 233 patients. Serum HCV RNA was not detectable with Quantiplex test in 39 patients; however, a low amount of serum HCV RNA was detected by the polymerase chain reaction technique in 15 patients. In the remaining 24 cases, no HCV RNA could be detected. Results were expressed as log of branched DNA. In a univariate analysis, serum HCV RNA was not correlated with sex, route of contamination, or genotype but was significantly correlated with present SRAC (at the time of the study) ($r = .26$, $P = .0001$) (Fig. 1).

A multivariate analysis (Table 2) was performed including all variables related to SRAC (age, sex, route of contamination, and genotype). The effect of SRAC on serum HCV RNA levels was independent of age, sex, and route of contamination ($P = .001$).

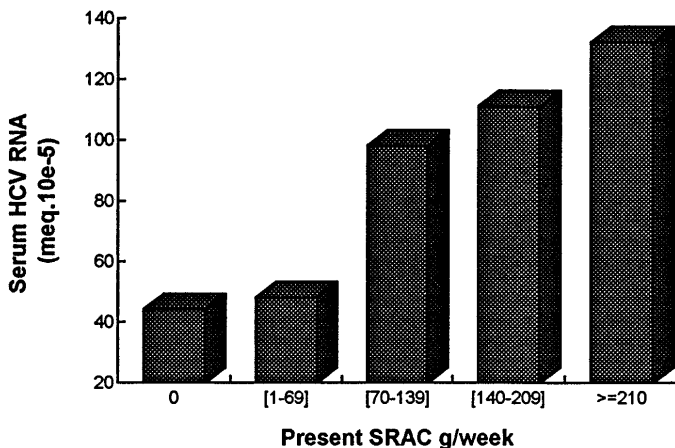


FIG. 1. Mean serum HCV RNA levels (branched DNA) according to SRAC expressed as grams per week. Present SRAC concerned a typical week during the month preceding HCV RNA measurement. Statistical significance: $r = .26$, $P < .0001$.

TABLE 2. Factors Correlated With Serum HCV RNA (log branched DNA): Uni- and Multivariate Analyses

Variables	Serum HCV RNA Log (branched DNA)	Univariate Analysis P	Multivariate Analysis* P
Age	0.10†	.14	.01
Past SRAC (g/day)	0.13†	.05	.16
Present SRAC (g/day):	0.26†	.0001	.001
Sex			
Male:	3.2 (1.6)‡	.13‡	.2
Female	2.9 (1.6)‡		§
Route of contamination			
Drug addicts	3.3 (1.8)‡	.40‡	.19
Transfusion	2.9 (1.5)‡		.35
Unknown	3.0 (1.5)‡		§
Genotype			
1a	3.6 (1.5)‡	.11‡	.52
1b	3.1 (1.4)‡		.07
2a	3.7 (1.5)‡		.88
3a	3.6 (1.5)‡		.61
Other	3.8 (1.4)‡		§

*Multivariate regression analysis.

†Correlation coefficient.

‡Comparisons of the means (sd) by ANOVA.

§Reference category.

Histological Results

Histological examination was performed in the 233 patients (Knodell score was available for 231 specimens). The biopsy was performed the day when serum samples were drawn for virological investigations. Liver lesions were graded and expressed according to the Knodell score. Results were expressed as fibrosis and activity scores according to the degree of periportal necrosis and of portal and lobular infiltration (Table 1). Fibrosis was graded as 0 in 40.7% of the samples and 1 in 43.3%. Cirrhosis (fibrosis 4) was found in 3.9%. Activity was mild in most cases as the total activity score was 5.4 (± 2.0 SD).

In univariate and multivariate analyses (Table 3) fibrosis was strongly related to age, ($P = .0001$) (Fig. 2). There was a significant correlation between fibrosis and SRAC before the diagnosis of chronic HCV infection, a variable assumed to reflect the whole amount of past alcohol intake ($P = .006$) (Fig. 3).

In a multivariate analysis, the total score of activity (mean 5.4) was related to age ($P = .03$). It was strongly related to past SRAC ($P = .0009$). It was related to the log serum HCV RNA ($P = .03$) and inversely related to present SRAC ($P = .04$).

We checked that the relation between activity index and log serum HCV RNA persisted in the group of patients with HCV RNA values $> .300$, caused by the discussion on reproducibility of the branched DNA test in low values.

DISCUSSION

In this cohort of patients with chronic HCV infection, we discovered the following: 1) a correlation between current alcohol consumption and serum HCV RNA levels, evaluated by the branched DNA test; 2) a correlation between hepatic fibrosis and the age of the patients; 3) a correlation between hepatic fibrosis and the past alcohol consumption; and 4) a correlation between serum of HCV RNA levels and the total histological activity index.

This cohort of 233 patients with chronic HCV infection

TABLE 3. Predictive Factors of Fibrosis (Uni- and Multivariate Analysis)

Variables	Score of Fibrosis (Knodell)				Univariate Analysis* P	Multivariate Analysis† P
	0	1	3	4		
Age: mean (sd)	38.2 (9.6)	40.6 (9.8)	47.4 (12.6)	52.9 (11.3)	<.0001	<.0001
Past SRAC (g/day): means (sd)	16.1 (29.0)	22.1 (36.6)	27.0 (43.1)	34.8 (45.4)	.02	.006
Present SRAC (g/day): mean (sd)	5.9 (13.8)	11.8 (22.6)	7.5 (21)	17.6 (48.6)	.25	.65
Sex						
Male %	59.6	69.0	60.7	77.8	.30	.29
Female %	40.4	31.0	39.3	22.2		
Route of contamination						
Drug addicts %	43.6	45.0	28.6	22.2	.90	.31
Transfusion %	21.3	25.0	42.9	44.4	.06	.15
Unknown %	35.1	30.0	28.5	33.4		
Log (bDNA): mean (sd)	2.7 (1.6)	3.4 (1.6)	3.4 (1.3)	3.6 (1.3)	.34	.08

*Univariate polychotomic logistic regression.

†Multivariate polychotomic logistic regression with a backward selection.

‡Reference category.

had epidemiological characteristics previously described in HCV carriers in Western countries²⁻⁵: approximately a third of contamination by blood transfusion, a third of iv drug users, and a third of so-called sporadic cases, a strong predominance of genotype 1 in the whole group and of genotype 3a in 20% of the cohort.

The major finding of the study is a highly significant correlation between SRAC and serum HCV RNA levels ($P = .0001$) This relation was similar with all genotypes. Previous studies show a correlation between cumulative alcohol consumption and viremia, evaluated semi-quantitatively.²⁰⁻²² However, the use of the questionnaire permitted the quantification of low alcohol intake and the detection of a dose-response relationship between the increase of HCV RNA levels and SRAC, even when alcohol consumption was moderate, i.e., as low as 140 g per week. However, a confounding factor cannot be excluded. The conditions of validity and reliability of the self-reported alcohol consumption questionnaires have been evaluated in different populations²³ and considered as biased only in alcoholic populations. Our study deals with moderate alcohol consumption (less than 3 drinks per day, i.e., 210 g per week) in 88% of the patients.

The pathogenic mechanisms of alcohol-induced increase

in serum HCV RNA in patients with HCV infection remain unclear. Both an increased release of HCV RNA into the serum, i.e., increased cell death or increased replication and delivery of virus from infected cells, or decreased clearance of viral RNA from the serum, i.e., alcohol induced impaired macrophage function, could be responsible.

The HCV RNA level is, in some studies, a predictive factor of a poor response to interferon therapy^{1-4,6}; thus, the effect of alcohol on serum HCV RNA could explain the previous observation that alcohol consumption is associated with a poor response to interferon therapy.^{7,21,24} A complete response was observed in 30% of nonhabitual drinkers and only in 6% of social drinkers.²¹ These findings can potentially have important practical implications on the following two topics: 1) preventing alcohol consumption in patients treated by interferon; and 2) paying particular attention to the subgroup of patients contaminated through iv drug use and who stopped drug addiction but converted to impulsive alcohol consumption.

The histological lesions observed in our patients were related to HCV infection, and the Knodell index could be determined in all patients but two. In this series of patients

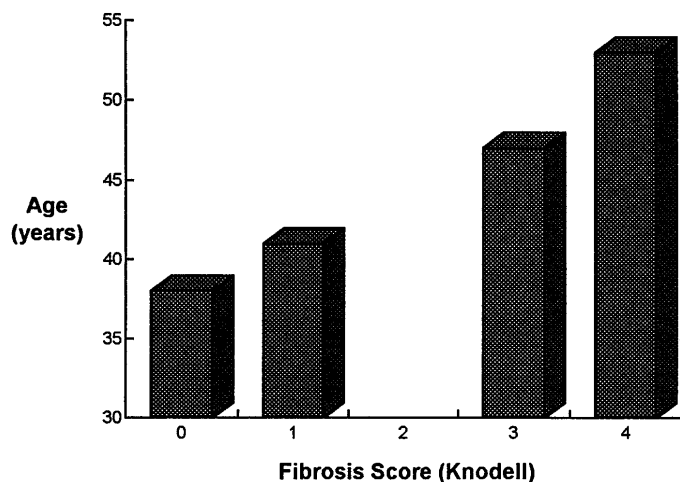


FIG. 2. Mean age of the patients and histological fibrosis expressed as Knodell index. Statistical significance: $P < .0001$.

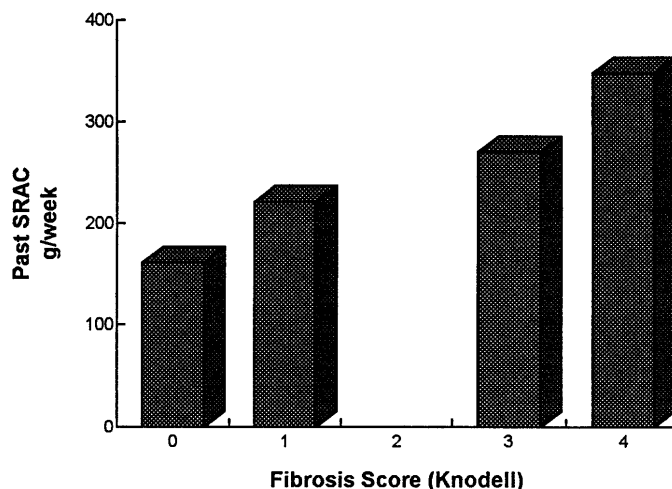


FIG. 3. Mean SRAC during the period preceding the diagnosis of HCV infection (expressed in grams per week), and histological fibrosis expressed with Knodell index. Statistical significance: $P < .02$ (univariate analysis).

with predominantly low alcohol consumption, we did not find frequent overlaps of histological lesions caused by alcohol and viral infections in contrast to others.^{11,12,25-28} We found, as others, a relation between fibrosis and age, and fibrosis and past alcohol consumption^{24,29-31} confirming the role of alcohol in inducing fibrosis. The relation of age with fibrosis was also previously documented in patients contaminated by blood products.³²⁻³⁴

We also found a statistical correlation between the histological activity index and log serum HCV RNA levels; however, our patients had mild histological activity with a total mean activity index of 5.4. An interaction between alcohol consumption and virus infection has been documented for other viruses. For example, alcohol intake has been shown to increase human immunodeficiency virus replication in human peripheral blood mononuclear cells in culture.¹³ The T lymphocyte system seems to be extensively affected by alcohol. There might be a transient immunosuppressive environment in which viruses (HIV and, possibly, HCV) replicate faster than in intact immune system. This hypothesis could explain both the increase of viral replication and the decrease of immune mediated liver lesions. Other situations of immunodepression are also characterized by the enhancement of viral replication (hepatitis B virus and human immunodeficiency virus) together with important lesions of fibrosis and mild histological activity. Moreover, cholestatic fibrosing lesions have been described with hepatitis B virus and hepatitis C virus infection in patients with a liver transplantation and treated with immunosuppressive agents.^{35,36} Chronic alcohol abuse has been shown in experimental models to induce a transient T suppressor cell dysfunction.^{37,38} It would indirectly release the regulatory control over the immune system and, therefore, act as a stimulant for CD4 lymphocytes.

In conclusion, the precise evaluation of SRAC, even with low values, in patients suffering from HCV chronic infection showed a clear relationship between the level of HCV RNA and alcohol consumption, suggesting a direct role of alcohol on HCV replication and/or HCV clearance. Histological lesions, particularly fibrosis, are related to age and to the past alcohol consumption. Although there was a statistical inverse correlation between SRAC and histological activity index, the very low variation of activity might be of questionable clinical significance. The practical importance of the strong relation between serum HCV RNA levels and fibrosis with past alcohol consumption should be emphasized, and patients suffering from HCV infection should be clearly advised to refrain from alcohol consumption intake as recommended by the recent consensus conferences in France³⁹ and in the United States.⁴⁰

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