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The relationships of chronic hepatitis and cirrhosis to alcohol intake, hepatitis B and C, and delta virus infection: a case-control study in Albania

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SUMMARY

The present study examined the effect of hepatitis B virus (HBV) and alcohol intake, and the role of hepatitis delta virus (HDV) and hepatitis C virus (HCV) in the aetiology of chronic liver disease in Albania. A total of 106 cases of liver cirrhosis or chronic hepatitis were compared to 195 control patients without these or other liver diseases. Adjusted odds ratios were 52·7 (95% CI 22·7–122) for HBV surface antigen, 26·9 (95% CI 4·9–147) for anti-HCV, 26·2 (95% CI 3·1–221) for anti-HDV, 2.4 (95% CI 1·3–4·4) for lifetime alcohol intake and 2·3 (95% CI 1–5·5) for duration of alcohol intake. Although not significant, an interaction was suggested between HBsAg and anti-HCV and between HBsAg and alcohol intake. Our study underlines the role of hepatitis viruses in the development of chronic liver diseases. Additionally, it suggests that heavy alcohol intake may magnify the effect of HBV on these diseases. HBV vaccination and alcohol abstention appear to be important strategies to reduce the risk of liver cirrhosis and chronic hepatitis in Albania.

INTRODUCTION

Infection with hepatitis B virus (HBV), hepatitis C virus (HCV), and alcohol intake are known risk factors for chronic liver disease [1–5]. HBV infection is endemic in Albania, with the prevalence of chronic carrier status for hepatitis B surface antigen (HBsAg) estimated as $> 10\%$. Delta virus (HDV) infection is reported in 6·2–12·7% of HBsAg positive subjects [6, 7], and HCV is found in 10% of patients with liver disease [8]. Data on alcohol intake in Albania are not available, but beer, wine, schnapps ('raki') and other beverages with a high alcohol content appear to be widely consumed.

Only very few studies have addressed the interaction between HBV and alcohol with regard to liver disease, thus making it important to replicate their findings [3, 9]. We took advantage of the high prevalence of these risk factors in Albania, to examine their joint effect. In addition, the study attempt to assess the roles of HCV and HDV in the aetiology of chronic liver diseases.

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STUDY POPULATION AND METHODS

A case-control study was conducted to examine the relationships of HBV, HCV and alcohol intake to chronic liver disease. Cases were patients with a diagnosis of chronic liver disease admitted in 1995 to the Liver Unit of the University Hospital Center (UHC) of Tirana, the most important centre for treatment of liver disease in Albania. Chronic liver disease included chronic hepatitis (high levels of transaminases at least 6 months prior to admission,

Table 1. *Distribution of cases and controls by gender, age, and educational level*

	Cases (%) $n = 106$	Controls (%) $n = 195$
Gender		
M	86 (81·1)	157 (80·5)
F	20 (18·9)	38 (19·5)
Age*		
< 20	2 (1·9)	7 (3·6)
20–29	11 (10·4)	30 (15·4)
30–39	23 (21·7)	37 (19·0)
40–49	28 (26·4)	59 (30·3)
50–59	21 (19·8)	37 (19·0)
60–90	17 (16·0)	18 (9·2)
⩾ 70	4 (3·8)	7 (3·6)
Education level (years)		
0–4	16 (15·1)	27 (13·8)
5–8	42 (39·6)	52 (26·7)
> 9	48 (45·3)	116 (59·5)

* The median (range) age for cases was 45 (16–80) years and for controls, 42 (14–80) years.

high γ-globulin levels, and/or diagnostic confirmation by biopsy), or liver cirrhosis (signs of portal hypertension or liver failure, and/or diagnostic confirmation by laparoscopy or biopsy). Primary or secondary biliary cirrhosis and autoimmune or metabolic liver diseases were excluded from cases. Controls were all patients admitted for the first time in 1995 to UHC on Mondays, Wednesdays and Fridays with the conditions listed in Table 1. Patients were excluded from the control group if they had the following diagnoses, or clinical or laboratory evidence of altered liver function at the time of admission: biliary tract disease, primary biliary cirrhosis, acute viral hepatitis, autoimmune, metabolic, alcohol or drug induced liver disease. Informed consent was obtained from each patient enrolled in the study. Consent rate for participation in the study was 100% for cases and controls.

Upon admission a standard pre-coded questionnaire was administered to cases and controls by two trained interviewers who were unaware of the study's objectives. Information was collected on age, sex, educational level, and alcohol intake as part of a dietary assessment. The average intakes of wine, beer and other alcoholic beverages were measured as ml/day, and subsequently transformed to grams of ethanol/day based on the average ethanol concentrations of beer (5%), wine (12%), and schnapps and other beverages with a high alcohol content (40%). The ages when the patient started and stopped drinking were recorded along with detailed information on alcohol consumption temporal changes.

The validity of the alcohol intake information was assessed by interviewing relatives, and estimating the concordance between the alcohol consumption reported by the subjects and that provided by their relatives. Agreement was found to be > 90%. The mean lifetime alcohol intake was calculated by multiplying the total daily ethanol consumption by the alcohol intake duration expressed in days. The mean lifetime daily alcohol intake was then computed. The 75th percentile of alcohol consumption in the control group considering only drinkers was used as a cut-off point for the lifetime and daily average intakes. The cases have been excluded from computation of cut points for alcohol parameters because the percentage of cases do not represent that of the general population. Furthermore we excluded non-drinkers to be more conservative. The cut-off points obtained are close to that used in the literature where the danger dose for liver disease is reported to be greater than 80 g alcohol daily [10]. For lifetime average intake, the 90th percentile was used.

Laboratory tests

Serum samples were stored at −20 °C. The presence of HBsAg, anti-HBc, and anti-HDV was ascertained by commercial kits: Monoclonal Auszyme, Ausab EIA, and Anti-Delta EIA (Abbott Diagnostics), respectively. HBsAg was tested in anti-HBc positive patients, and anti-delta in HBsAg positive patients. Anti-HCV status was assessed by the third generation ELISA (Ortho Diagnostics), and confirmed by third generation RIBA (Chiron Corporation).

Statistical analysis

Adjusted odds ratios were estimated, by multiple logistic regression. SPSS, version 6.0 [11], was used for statistical analysis. The statistical significance of additive and multiplicative interactions was assessed as suggested by Schlesselman [12], and by a homogeneity test, respectively [13].

RESULTS

The study included 106 cases and 195 controls. Seventy-four cases were newly diagnosed, and 32 had been admitted previously for chronic liver disease. In all but two cases with chronic hepatitis the diagnosis

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Table 2. *Distribution of cases and controls by diagnosis*

Cases	*n* (%)	Controls	*n* (%)
Liver cirrhosis	84 (79·2)	Infectious disease (influenza, urinary tract infection, erysipelas infection, diphtheria, respiratory tract infection, brucellosis)	31 (15·9)
Compensated	32		
Decompensated	52		
Chronic hepatitis	22 (20·8)		
Active	14		
Persistent	8	Occupational disease (pneumoconiosis, phenolic intoxication, Pb, intoxication)	49 (25·1)
		Dermatologic disease (eczema, dermatitis, acne)	35 (17·9)
		Eye, ear, nose and throat disease	37 (19·0)
		Other (first myocardial infarction attack, allergic diseases, appendicitis, inguinal hernia)	43 (22·1)

Table 3. *Distributions of cases and controls according to selected viral and alcohol intake markers, with corresponding odds ratios*

Risk factor	Cases (%)	Controls (%)	$OR_{unadjusted}$ (95% CI)	$OR_{adjusted}$ (95% CI)
HBsAg+	83 (78·3)	23 (11·8)	27·0 (14·3–50·9)	52·7* (22·7–122)
anti-HBc+	103 (97·2)	132 (67·7)	16·4 (5·0–53·7)	17·6* (5·1–60·6)
anti-HCV+	13 (12·6)	3 (1·5)	9·2 (2·6–33·3)	26·9† (4·9–147)
anti-delta+	10 (9·4)	1 (0·5)	20·2 (2·5–160)	26·2* (3·1–221)
Mean daily alcohol intake (LTDAI) > 67 g	21 (19·8)	11 (5·6)	4·1 (1·9–9·0)	2·4‡ (1·3–4·4)
Duration of alcohol intake (DAI) > 20 years	7 (6·6)	4 (2·1)	3·4 (1·0–11·8)	2·3‡ (1·0–5·5)
Lifetime alcohol intake (LTAI) > 595 g	17 (16·0)	5 (2·6)	7·3 (2·6–20·3)	2·6‡ (1·3–5·5)

* Adjusted for sex, age, educational levels, LTDAI anti HCV.
† Adjusted for, sex, age, educational levels, LTDAI, and HBsAg.
‡ Adjusted for sex, age, educational levels, anti HCV and HBsAg.

was substantiated by biopsy. As seen in Table 1, cases and controls had similar age distributions, but cases seemed to have less formal education than controls. Eighty-four of the 106 cases (80%) had liver cirrhosis. Among controls, approximately 25% were admitted for occupational diseases such as pneumoconiosis. Other diagnoses were ear, nose and throat problems (19%), skin disorders (18%) and infections (16%) (Table 2).

The adjusted ORs are shown in Table 3. The adjustments were performed only for the variables not intercorrelated. Strong associations were observed between all viral markers, particularly HBsAg, and chronic liver disease. In addition using those individuals, negative for both HBsAg and delta virus as the reference category, the adjusted OR associated with HBsAg was approximately 54·7 in the absence of delta, and 250·0 in its presence. It is not possible to fully assess interaction between HBsAg and delta virus because the latter is present only in individuals with HBV.

Using the selected cut-off points, markers of alcohol use were associated with an approximate doubling of chronic liver disease odds (Table 3). These associations seemed to follow a graded pattern, as exemplified by the trend related to average lifetime daily alcohol intake shown in Table 4 (trend test, $P = 0{\cdot}0036$).

Although none of the interactions was found to be statistically significant, it is of interest that for HBsAg and anti-HCV (Table 5), the joint effect appeared to be less than multiplicative, but more additive (observed joint OR = 195·0; expected joint OR: multiplicative model = 479·4, additive model = 47·1). Both multiplicative and additive interactions are suggested by

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Table 4. *Distributions of cases and controls according to selected categories of lifetime alcohol intake, with corresponding odds ratios*

Mean daily alcohol intake	Cases	Controls	$OR_{unadjusted}$ (95% CI)	$OR_{adjusted*}$ (95% CI)
0–67 g	85	184	1·0	1·0
68–105 g	4	7	1·2 (0·3–5·0)	3·6 (0·6–21·4)
> 105 g	17	4	9·2 (2·9–38·4)	8·6 (1·8–40·8)
P for trend	—	—	—	0·0036

* Adjusted for sex, age, educational levels, HBsAg and anti-HCV.

Table 5. *Distribution of cases and controls according to combinations of categories of HBsAg and anti-HCV, and of HBsAg and lifetime daily alcohol intake (LTDAI)*

Risk factor	Cases	Controls	$OR_{unadjusted}$ (95% CI)
HBsAg/LTDAI†			
Negative/0–67 g	20	161	1·0
Negative/> 67 g	3	11	2·2 (0·4–9·3)
Positive/0–67 g	65	23	22·8 (11·2–47·0)
Positive/> 67 g	18	0	291* (16·9–5021)
HBsAg/anti-HCV†			
Negative/negative	16	169	1·0
Negative/positive	4	3	14·1 (2·1–102)
Positive/negative	74	23	34·0 (16·1–72·7)
Positive/positive	9	0	195* (10·9–3506)

* To compute this OR we added 0·5 to each of the four related cells.
† Both multiplicative and additive interactions were not statistically significant.

examination of OR point estimates for HBsAg and daily alcohol intake greater than 67 g (observed joint OR = 291; expected joint OR: multiplicative model = 50·2, additive model = 24). Because of small numbers, the interaction between HCV and alcohol intake could not be assessed.

When only cirrhotic cases were considered in the analysis, the ORs were similar to those reported in Table 3. Furthermore, the OR point estimates did not change materially when only newly diagnosed cases were analysed.

DISCUSSION

In the present study, HBV and HCV were found to be strongly and significantly associated with liver cirrhosis and chronic hepatitis.

The very high OR for HBsAg was consistent with previous findings [2]. A large HBV diffusion was suggested by its high prevalence among the controls; this prevalence estimate was similar to that found in a recent study [7], but lower than that found among Albanian refugees [14], a discrepancy likely explained by the lower socio-economic level of the refugees. These figures are different from those reported in other European countries, where HCV appears to be the principal aetiologic agent for chronic liver disease [15].

The presence of anti-delta appeared to strengthen the association between HBsAg and chronic liver disease, confirming findings of previous studies that delta infection worsens prognosis in HBsAg chronic carriers [16, 17].

The joint presence of HBV and HCV appeared to interact, albeit not significantly, in an additive fashion, consistent with a recent case-control study carried out in Taiwan [2]. Also consistent with previous findings [4, 5], we found alcohol consumption to be associated with liver disease and the association increased in a graded manner (Table 4). Although the OR for average daily alcohol intake > 67 g was lower than that related to hepatitis viruses, our data suggest the presence of both additive and multiplicative positive interactions between alcohol consumption and HBV. Unfortunately, because of the small numbers, it was not possible to assess the interaction between HCV and alcohol intake, as recently suggested [18].

In the present study, cases and controls were recruited from the same hospital in an attempt to prevent selection bias. Additionally they were questioned by the same interviewers, who were unaware of the study's main objectives, and of their case-control status. Moreover, the information on alcohol intake was collected as part of an overall dietary assessment, thus, further preventing differential information bias. Our results are generally consistent with those of previous studies. On the other hand, because viral antigens and antibodies were measured after the case and control diseases had started, the associations

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found may be the result of temporal biases. In order to avoid such biases, future studies should use nested case-control designs.

Because HBV is the major cause of liver diseases in Albania, the mandatory use of vaccination as well as prevention and cessation of heavy alcohol intake appear to be sound primary prevention strategies.

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