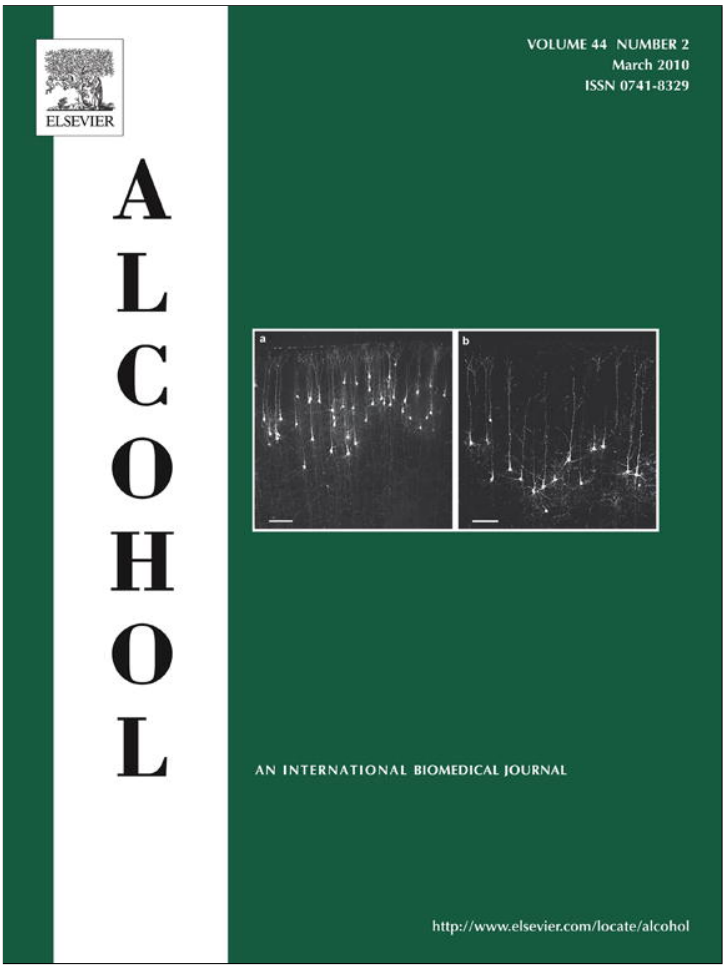

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Alcohol consumption among patients with hepatitis B infection in northern Portugal considering gender and hepatitis B virus genotype differences

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Abstract

Alcohol abuse is an important public health problem. In Portugal with a population of 10 millions of inhabitants, there are around 10% of alcoholics or excessive alcohol drinkers and 1% of chronically infected patients with hepatitis B virus (HBV). To examine the characteristics of patients with higher levels of alcohol consumption and to investigate the association between alcohol consumption and liver damage a total of 298 chronically infected individuals, with HBV genotyped and submitted to liver biopsy, were classified with Child's grading and separated by habits of alcohol intake, less and greater than 20 g/day. No significant differences were observed about genotype but genotypes A and D were predominant in both of them. A higher percentage of males ($P < .001$) were observed in the group with alcohol intake above 20 g/day, as well a lower proportion of patients with HBeAg negativity ($P \leq .035$). In this group, biochemistry parameters, such as alanine aminotransferase ($P = .006$), aspartate aminotransferase ($P = .001$), gamma-glutamyl transferase ($P < .001$) were elevated in a significantly higher proportion than in the other group. The analysis of hematological parameters showed significantly lower values of platelets ($P = .042$) and mean corpuscular volume ($P < .001$) and significantly higher values of prothrombin time ($P < .001$) in the group with higher levels of alcohol consumption. The characteristics of biopsy ($P < .001$) and Child–Phug's classification ($P = .002$) revealed more severe results in this group. Logistic regression showed a positive association between liver damage and alcohol intake, increasing with age. In female patients, a strong positive association between alcohol intake and liver damage was also found (odds ratio: 9.379; 95% confidence interval: 0.859–468.422; $P = .037$); however, the most severe cases were only observed in women older than 45 years. In patients with HBV infection, alcohol is associated with a more severe liver disease. No evidence was found concerning association with HBV genotype.

Keywords: Hepatitis B; Genotypes; Alcohol consumption; Liver damage; Northern Portugal; Gender

Introduction

Alcohol abuse is an important public health problem with a significant morbidity and mortality (Gramenzi et al., 2006). Alcohol consumption in Portugal is 15% higher than in the rest of Europe. It is estimated that there are approximately 1 million alcoholics or excessive alcohol drinkers in Portugal (WHO, 2008b). A study conducted in 2003 by World Health Organization (WHO) showed an annual pure alcohol consumption of 9.49 liters per capita (WHO, 2008a). A Portuguese study confirmed that there are around 1 million of alcoholics or excessive alcohol drinkers (Pinto, 2000). According to WHO, in Portugal in the year 2004 the standardized death rate for chronic liver disease and cirrhosis, all ages, per 100,000 was 13.31 (WHO, 2009). The fact that Portugal had such a high liver disease–related mortality is not surprising, since Portugal was the fifth alcohol consumer in the world (Spirits, 2002).

Several epidemiologic studies suggest that chronic alcoholics are at risk of viral infections. Clinical and basic research has demonstrated that alcohol not only worsens

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the natural history of chronic viral hepatitis, like hepatitis B virus (HBV) but also seems to interact with the viral replication cycle leading to an unusual serum virologic profile and/or modification in the serum concentration of viral particles (Nalpas et al., 1998).

HBV is a major cause of hepatocellular carcinoma (HCC) in the world (Wang et al., 2003). The infection with HBV and its interaction with alcohol consumption are very well documented, and it is possible to verify that the progression to more severe liver disease is very rapid and aggressive. In a physiological way, it was demonstrated that several signals activated by HBV X protein are potentiated cooperatively by alcohol, including tumor necrosis factor-alpha and ethanol-induced apoptosis by a caspase-3-dependent mechanism. Those mechanisms could be implicated in the synergistic effect of alcohol drinking and viral hepatitis on liver injury (Gao, 2002). In the transgenic mice model, viral replication is enhanced by alcohol consumption (Larkin et al., 2001), suggesting that alcohol upregulates HBV gene expression and replication. This would promote not only higher viral loads but also trigger an antiviral immune response leading to severe bouts of liver disease.

Several studies (Saunders et al., 1983) have shown that patients with alcoholic cirrhosis showed evidence of past or current infection with HBV more commonly than did healthy nonalcoholic subjects (Bassendine et al., 1983; Goudeau et al., 1981; Inoue, 1977; Mills et al., 1981). A longitudinal study of hepatitis B surface antigen–positive healthy blood donors tested in Japan between 1972 and 1975 found that alcohol consumption greater than 27 g/day (duration of alcohol use not stated) was associated with more than a fivefold increase in the relative risk for development of HCC (Oshima et al., 1984). A study that compared two case series suggested that patients with HCC caused by chronic HBV and chronic alcohol are approximately 10 years younger than patients with HCC caused by chronic HBV alone (Ohnishi et al., 1982; Pereira et al., 1994).

A case–control study, performed in Albania, showed that individuals with HBV infection and excessive alcohol intake had an increased probability (odds ratio [OR]: 26.9; 95% confidence interval [CI]: 4.9–147) of developing chronic hepatitis and cirrhosis when compared with patients without these or other liver disease. Although not significant, interaction was suggested between HBsAg and alcohol intake (Kondili et al., 1998). A study among the Portuguese population also found that alcohol intake was the most important risk factor to HCC, with a frequency of 5.2% in patients with alcoholic hepatic cirrhosis (Oliveira et al., 2001).

The sudden increase in alcoholic liver disease among women showed their susceptibility to the hepatotoxic effects of alcohol. Women tended to present more severe liver disease than men, particularly alcoholic hepatitis, and did so after a shorter period of excessive drinking and at a lower daily alcohol intake. Differences in body size and composition may be partly responsible for the greater susceptibility of women but differences in immune reactivity between the sexes may also play a part (Saunders et al., 1981).

The aim of this study was to assess the characteristics of patients with hepatitis B infection with higher levels of alcohol consumption in the northern region of Portugal, and to investigate the association between alcohol consumption and liver damage, considering gender and genotype differences.

Material and methods

Patients

Most of the patients with hepatitis B infection (documented because of the presence of HBsAg in serum for at least 6 months) from the North of Portugal are treated in three hospitals in the city of Oporto: Hospital de São João, Hospital de Santo António, and Hospital Joaquim Urbano. The patients of this study were observed in the Hospital de Santo António and Hospital Joaquim Urbano. The study was approved by the Health Ethics Committee of Hospital de Santo António, and written consent was obtained from all participants. HBV genotypes were determined in 298 patients using a molecular method. Histologic evaluation was performed in all of them. Only individuals who were older than 16 years were included in this study. In Portugal, since 2002 the law forbids sale of alcoholic drinks to individuals less than 16 years.

Clinical findings

Information relating to patient demographics, biologic, histologic, and medical history was recorded from medical files. Demographic characteristics include the following: age, gender, place of birth, and residence, as well as marital status. The presumed sources of HBV infection were recorded. According to information provided by the physicians, this classification is based on the patient clinical story and the answers to a set of questions. The presumed source of HBV infection is classified as sexual transmission when the physician has no doubt about this source. The intrafamilial transmission presumed source is considered when transmission seems to occur inside the family group but not necessarily by sexual way, and there is no evidence of other ways of transmission.

Alcohol consumption was also evaluated and categorized as less than 20 g/day and greater than 20 g/day, based on the WHO classification and other important references in this area (Anderson, 1996a, b; Dawson, 2000; Fiellin et al., 1998).

Detection of virologic markers in serum

The study population was tested routinely for HBsAg, anti-HBs, and anti-HBc. HBeAg and anti-HBe antibody were also evaluated using Vitros ECI (Ortho-Clinical Diagnostics, Amersham, Buckinghamshire, UK).

HBV DNA quantification

HBV DNA levels were determined using VERSANT® HBV DNA 3.0 assay (bDNA) (Bayer, Tarrytown, NY, USA), which is a signal amplification nucleic acid probe assay for the direct quantification of human hepatitis B viral DNA in HBV-infected patients. The quantification range of the assay is from 2,000 to 100,000,000 HBV DNA copies/mL. Values below the lower quantification limit are reported as "<2,000" HBV DNA copies/mL noted as "virus not detected."

HBV genotyping

HBV genotypes were determined by the TRUGENE® HBV genotyping kit. When used in conjunction with OpenGene® DNA Sequencing System (Bayer) the kit provides a method to obtain bidirectional sequence from overlapping surface antigen and from domains B to E of reverse transcriptase region of HBV. The HBV DNA sequences are queried against a library of known genotypes and mutations to deliver a TRUGENE HBV genotyping report.

Liver enzymes and other blood parameters

Numerous biochemical tests that detect abnormal liver function can help in the confirmation of the presence of liver disease but cannot define the cause. The most common blood tests that assess liver function include the following: alanine aminotransferase (ALT) with reference values of 10–36 U/L at 37°C and aspartate aminotransferase (AST) with reference values of 10–30 U/L at 37°C, which are contained within the liver cells. Inflammation of the liver causes these enzymes to be released in the blood in abnormally high amounts. Blood levels of these two enzymes are roughly elevated in proportion to the degree of liver damage. The other blood test performed for detection of eventual liver damage was gamma-glutamyl transpeptidase (GGT) with reference values of 10–66 U/L at 37°C, alkaline phosphatase (AP) with reference values of 45–122 U/L at 37°C, and alpha-fetoprotein with reference values less than 10.9 ng/mL. To investigate liver enzymes, elevated levels were separated from normal levels. All parameters were performed with Cobas Integra 800 (Roche, Mannheim, Germany).

Hematological parameters like mean corpuscular volume (MCV) and platelets were also evaluated, with normal levels respectively, between 80 and 100 fL and between 150 and 400×10^9/L; both evaluated with Advia 120 (Bayer). Prothrombin time (PT) was performed in ACL TOP (Instrumentation Laboratory Company, Lexington, MA, USA).

Liver biopsy

In the group of individuals with information about histologic lesions, the following characteristics of liver disease were evaluated: necroinflammatory, fibrosis, and esteatosis.

Child–Pugh classification

Assessment of liver damage was evaluated when genotyping was performed. Child's grading of disease severity in chronic liver disease was classified with Pugh's 1973 modifications (Pugh et al., 1973) based on information recorded from medical files. This classification has three grades, from A to C: grade C is the severest, corresponding to an overall 5-year mortality of 88%, grade B to 38%, and grade A to 29%. Because this classification is used to assess the prognosis of chronic liver disease, mainly cirrhosis, in these studies, three categories were considered to evaluate liver damage in the studied population: (1) Child grade C, (2) Child grade B, and (3) others, including patients classified as Child grade A and patients without liver disease reported.

Statistical analysis

The distribution of viral load and PT was logarithmically transformed. The results are given as geometric means with 95% CI. For the other quantitative variables, results are given as arithmetic means with 95% CI. *t*-Test for independent samples was used to compare the groups defined as alcohol intake under or above 20 g/day. Qualitative variables were described as percentage and compared using the Pearson chi-square test (with continuity correction) as appropriate or by Fisher exact test. Variability in the number of participants included in the analyses is because of incomplete data sets.

The association between alcohol intake and liver damage was analyzed separately for male and female patients.

For the male patients, the statistical analysis of the association between alcohol intake and the binary outcome (Child's grade equivalent C or B vs. others, including patients classified as Child grade A and patients without liver disease reported, as reference) was performed using logistic regression analysis. Two models were created for the regression analyses. The crude model consisted only of the dependent variable and alcohol intake group as independent variable, whereas in the adjusted model the effect of alcohol intake was corrected for age. Hepatitis B genotype was not included because of not being statistically significant. To analyze the possible difference in the association between the outcome and alcohol intake with age, an interaction term was added. For the interaction term a P value lower than .10 was considered to be significant. The continuous variable modeling of age was tested through the fractional polynomial method; a procedure that makes use of the full information available in the data when a linear relationship is not assumed (Royston and Altman, 1994). Fractional polynomial modeling confirmed that the association with age was linear. The Hosmer–Lemeshow goodness of fit test was used to check the lack of fit of the final logistic model.

In female patients, only five cases were classified as Child's grade equivalent C or B. Because of this reduced number, the association between alcohol intake and the binary outcome, Child's grade equivalent C or B versus

Child's grade equivalent A, and patients without liver disease reported, was analyzed considering Fisher exact test and an exact method to construct confidence limits for OR. Female patients were divided according to their median age (45 years old) to analyze the possible difference in the association between the outcome and alcohol intake with age.

Statistical analysis was performed with SPSS (version 16.0) and STATA (version 9.0) and two-sided significance of 5% was used throughout.

Results

Table 1 shows a description of the total studied sample ($n = 298$) by alcohol intake levels: 52.0%, less than 20 g/day ($n = 155$) and 48.0% greater than 20 g/day ($n = 143$). No significant differences between the study groups were observed with respect to genotype distribution and age. Genotypes A and D were predominant in both groups of individuals. However, a significantly higher percentage of males ($P < .001$) were observed in the group

Table 1
Characteristics of individuals according to alcohol intake

	Alcohol intake <20 g/d ($N = 155$)		Alcohol intake >20 g/d ($N = 143$)		
Chacteristics	*n*	(%)	*n*	(%)	*P*
Genotypes					.910[a]
A	50	(32.3)	45	(31.5)	
C	1	(0.6)	1	(0.7)	
D	92	(59.4)	88	(61.5)	
E	5	(3.2)	7	(4.9)	
F	7	(4.5)	2	(1.4)	
Gender					<.001
Female	86	(55.5)	41	(28.7)	
Male	88	(44.5)	102	(71.3)	
Age mean (95% CI)	43.4	(41.0–45.7)	45.3	(43.1–47.5)	.231
Routes of transmission					.048
Perinatal	41	(26.5)	30	(21.0)	
Sexual	35	(22.6)	21	(14.7)	
Intrafamilial	45	(29.0)	42	(29.4)	
Unknown	34	(21.9)	50	(35.0)	
Viral markers					
Anti-HBc positive	154	(99.4)	142	(99.3)	1.000
HBeAg negative	103	(66.5)	118	(82.5)	.002
Anti-HBe positive	101	(65.2)	110	(76.9)	.035
Viral load[b] geometric mean (95% CI)	638,450.9	(349,654.2–1,165,778.9)	306,753.6	(161,280.4–583,500.2)	.101
Biochemistry parameters greater than reference values					
ALT	76	(49.7)	94	(66.2)	.006
AST	66	(42.9)	92	(64.8)	.001
AP	6	(4.0)	16	(11.5)	.028
GGT	20	(13.4)	52	(37.4)	<.001
Alpha-fetoprotein	6	(4.6)	11	(8.7)	.284
Characteristics of biopsy					<.001
Necroinflammatory	11	(15.7)	31	(21.7)	
Fibrosis	50	(71.4)	31	(21.7)	
Esteatosis	9	(12.9)	81	(56.6)	
Hematological parameters					
Platelet $\times 10^9$/L mean (95% CI)	194.3	(182.7–205.9)	175.3	(160.6–189.9)	.042
MCV mean (95% CI)	92.3	(91.2–93.6)	86.7	(85.2–88.3)	<.001
PT geometric mean (95% CI)	12.7	(12.4–13.0)	14.0	(13.5–14.5)	<.001
Child–Phug's classification					.002[c]
Grade C	1	(0.7)	4	(3.3)	
Grade B	4	(3.0)	15	(12.3)	
Others[d]	130	(96.3)	103	(84.4)	

CI = confidence interval; ALT = alanine aminotransferase; AST = aspartate aminotransferase; AP = alkaline phosphatase; GGT = γ-glutamyl transpeptidase; MCV = mean corpuscular volume; PT = prothrombin time.

[a] Evaluated only with genotypes A and D.

[b] Evaluated only for patients with viral load between 2,000 to 100,000,000 hepatitis B virus DNA copies/mL.

[c] Evaluated considering Child's grade equivalent C or B versus others.

[d] Including patients classified as Child grade A and patients without liver disease reported, as reference.

with alcohol intake above 20 g/day. The distribution of the presumed sources of transmission of viral infection showed a marginal significant difference between the two groups of alcohol intake ($P = .048$).

The proportion of individuals with HBeAg negativity and anti-HBe positivity were significantly higher in the group with alcohol intake above 20 g/day ($P \leq .035$); however, the proportion of patients with anti-HBc was similar in the two groups of alcohol intake levels.

Biochemistry parameters like ALT ($P = .006$), AST ($P = .001$), GGT ($P < .001$) were elevated in a significantly higher proportion of patients in the group with an alcohol intake above 20 g/day compared with the group with an alcohol intake under 20 g/day.

Comparison between individuals with alcohol intake levels less than 20 g/day and greater than 20 g/day showed significant differences for the frequency of characteristics of biopsy ($P < .001$), with a panel of more severity in individuals with an alcohol intake above 20 g/d.

Evaluation of hematological parameters, showed significantly lower values of platelets ($P = .042$) and MCV ($P < .001$) and significantly higher values of PT ($P < .001$) in the group with the higher alcohol consumption level compared with the group with the lower level of alcohol consumption.

Child–Phug's classification showed different results for both groups, with more severe results for the group with an alcohol intake above 20 g/day ($P = .002$).

For male patients, logistic regression was used to study the relationship between alcohol intake and liver damage (Table 2). The outcome was Child's grade equivalent C or B versus others, including patients classified as Child grade A and patients without liver disease reported, as reference. Alcohol intake was classified as greater than 20 g/day and less than 20 g/day as reference. For male patients, a positive marginal significant association was found between liver damage and alcohol intake (OR: 2.979; 95% CI: 0.938–9.462; $P = .064$). Significant changes were observed as a result of the correction for potential confounders (corrected model). Results of the multiple logistic regression analysis showed a significant positive interaction term in the model, implying that the effect of higher levels of alcohol consumption increases with age. The interpretation of the variable "alcohol intake" is not so simple because of the interaction term with age. To achieve a better understanding, we calculated the estimated ORs for the effect of alcohol intake in achieving a more severe liver disease, for different ages (Table 3). The strength of the association between alcohol intake and liver damage seems to increase with the age of the patient.

Table 2
Effect of alcohol intake on the severity of liver disease according to Child–Pugh classification[a] with logistic regression

Characteristics	OR	95% CI		P
Male gender				
Crude model:				
Alcohol intake (<20 g/d as reference)	2.979	0.938	9.462	.064
Adjusted model:				
Alcohol intake (<20 g/d as reference)	0.023	0.000	1.488	.076
Age	0.990	0.925	1.060	.778
Interaction: alcohol intake × age	1.106	1.015	1.206	.021
Female gender				
Alcohol intake (<20 g/d as reference)[c]	9.379	0.859	468.422	.037
Less than 45 years:				
Alcohol intake (<20 g/d as reference)	[b]			
Greater than 45 years:				
Alcohol intake (<20 g/d as reference)[c]	8.000	0.681	406.695	.062

OR = odds ratio; CI = confidence interval.

[a] Child grade equivalent B and C versus others, including patients classified as Child grade A and patients without liver disease reported, as reference.

[b] Not estimated because of all cases being Child grade equivalent A or without liver disease reported.

[c] Analyzed with Fisher exact test and an exact method to construct confidence limits.

Table 3
Estimated OR by age for the effect of alcohol intake on the severity of liver disease according to Child–Pugh classification[a] in male patients

Male age (y)	OR
35	0.557
45	1.384
55	3.438
65	8.542
75	21.221

OR = odds ratio.

[a] Child grade equivalent B and C versus others, including patients classified as Child grade A and patients without liver disease reported, as reference.

In female patients (Table 2) a strong positive association between alcohol intake and liver damage was also found (OR: 9.379; 95% CI: 0.859–468.422; P: .037). When women were divided into two groups according to their median age, in the group with ages older than 45 years, no significant changes in the estimate of the OR were observed. However, no cases classified as Child's grade B or C were observed in the younger group.

Discussion

Alcohol, the only cause of alcoholic liver disease, is also a known hepatotoxic agent, which may exacerbate liver injury caused by other agents. The wide prevalence of alcohol use and abuse in society makes it an important cofactor in many other liver diseases. Examples of liver diseases that are significantly influenced by ingestion of alcohol include chronic viral hepatitis (Balasubramanian and Kowdley, 2005).

HBV and hepatitis C virus (HCV) are the two most frequent causes of chronic hepatitis worldwide and lead to most cases of end-stage liver disease and HCC (Feld and Liang, 2006; Weisberg et al., 2007). Because of their high prevalence in the general population, alcohol use and abuse are often associated with hepatitis virus infections, and in

these cases, it has been demonstrated that alcohol plays a role as a comorbid factor in the development of liver damage and disease progression (Gramenzi et al., 2006). Moreover, alcohol abuse in patients with chronic hepatitis B is associated with increased risk of cirrhosis and HCC (Corrao et al., 1998; Larkin et al., 2001). Marcellin et al. (2008) found a strong association between alcohol consumption and mortality in these patients.

In this study, the association between alcohol intake and liver damage was analyzed separately for male and female patients.

No significant differences between the study groups with alcohol intake levels under and above 20 g/day were observed with respect to genotype distribution and age. Genotypes A and D were predominant in both group of individuals. This distribution is similar to other European studies that confirm the predominance of these two genotypes, particularly in Mediterranean countries (Basaras et al., 2007; Echevarria and Leon, 2004; Sanchez-Tapias et al., 2002). In our group of individuals, no relationships between HBV genotypes and the presence of extrahepatic manifestations were evidenced in patients with chronic HBV infection. In India, genotype D was reported to be associated with more severe liver disease than genotype A and that may predict occurrence of HBV HCC in young patients (Kao, 2002). Other European groups (Cacoub et al., 2005) in this area of investigation found similar results to the Portuguese study, and the clinical significance of HBV genotypes remains largely unknown.

In the group above 20 g/day of alcohol intake, a significantly higher percentage of males ($P < .001$) were observed. This predominance of male gender is in accordance with other studies (Livingston and Room, 2009). Males drank at higher levels than females and behaved more problematically. However, women are less likely to be suspected of alcohol abuse, even if they develop withdrawal symptoms in hospital. There are several reasons for this. Because of social stigma, women are less likely to admit alcohol abuse, and they have also more opportunities to "cover up." Women can drink when they are at home alone, without their relatives noticing this behavior (Morgan and Sherlock, 1977).

When the evaluation between routes of infection for viral hepatitis was performed, the distribution of the presumed sources of transmission of viral infection showed a marginal significant difference between the two groups of alcohol intake ($P = .048$). The alcohol intake is also a cofactor in severity of disease, but it probably does not affect the route of hepatitis infection. These results are in concordance with other descriptive epidemiologic studies of chronic hepatitis B infection, particularly in countries of Southern Europe (Stefos et al., 2009).

The proportion of individuals with HBeAg negativity and anti-HBe positivity was significantly higher in the group with alcohol intake above than 20 g/day ($P \leq .035$); however, the proportion of patients with anti-HBc was similar in the two groups of alcohol intake levels. These findings show probably the absence of *e*-antigen (presumably indicating precore or core promoter mutation). These results also confirm other epidemiologic observations that show a higher frequency of HBV serologic markers in chronic alcoholics compared with the general population. This may be the result of an increased susceptibility of alcoholics to infection and/or to an ethanol-mediated stimulation of HBV gene expression and replication.

The biochemical tests considered, ALT, AST, GGT and AP do not support on its own the diagnosis of alcoholism. Sensitivities and specificities vary considerably and depend on the population concerned. GGT continues to remain the test that combines the greatest convenience and sensitivity; its diagnostic accuracy can be enhanced by combination with other traditional markers (AST, ALT, and MCV). Biochemistry parameters like ALT ($P = .006$), AST ($P = 0.001$), and GGT ($P < .001$) were elevated in a significantly higher proportion of patients in the group with an alcohol intake above 20 g/day, compared with the group with an alcohol intake less than 20 g/day. Evaluation of hematological parameters showed significantly lower values of platelets ($P = .042$) and MCV ($P < .001$) and significantly higher values of PT ($P < .001$) in the group with the higher alcohol consumption level compared with the group with the lower level of alcohol consumption. All these results seem to be in accordance with many other studies, particularly in the group of individuals with elevated alcohol intake (Larkin et al., 2001; Mann et al., 2003).

Comparison between individuals with alcohol intake levels less than 20 g/day and greater than 20 g/day showed significant differences for the frequency of characteristics of biopsy ($P < .001$), with a panel of more severity in individuals with an alcohol intake greater than 20 g/day. These results are in accordance with the results from Child–Phug's classification with more severe results for the group with an alcohol intake greater than 20 g/day ($P = .002$) and other studies in this area including Portugal (Anttila et al., 2005; Donohue, 2007; Hoek and Pastorino, 2004; Pereira et al., 1994). These results suggest that the combination of alcohol abuse and HBV infection increases the histologic evidence of liver damage.

Liver damage was evaluated using Child's grading with Pugh's modifications. The study of the relationship between alcohol intake and liver damage in male patients suggests that the effect of higher levels of alcohol consumption increases dramatically with age (Table 3). Age and alcohol were also reported as risk factors to HCC in a large cross-sectional study performed in Korea and in many others (Chung et al., 2007; Oliveira et al., 2001; Pereira et al., 1994; Wang et al., 2003).

The reduced number of female patients classified as Child's grade B or C did not allow the analysis of the association of alcohol intake with liver damage adjusted for age. However, when women were divided in two groups, according to their median age, cases classified as Child's grade B or C were observed only in the older group,

suggesting an increase of the association between liver disease and alcohol with the age of the patient.

Other studies found that women tend to present more severe liver damage, particularly alcoholic hepatitis, and do so after a shorter period of excessive drinking and at a lower daily alcohol intake. Differences in body size composition, and the role of estrogens are responsible for the greater susceptibility of women, but differences in immune reactivity between the sexes may also play a part (Saunders et al., 1981). In this study, the comparison between genders of the strength of the association between alcohol and liver damage was not possible. However, for both genders, this association seems to increase with age.

Hepatitis B infection might influence the development of liver disease in heavy drinkers in several ways. Firstly, persistent infection with HBV might potentiate alcohol-induced liver damage, and there is evidence that this may occur at fairly low alcohol intakes (Villa et al., 1982). Secondly, chronic active hepatitis associated with HBsAg may coexist with alcoholic liver disease, and the two disease processes would probably result in more rapid progression to cirrhosis.

On the one hand, infections with HBV and HCV are a major risk for the development of HCC in excessive drinkers who should be protected against these viruses (Nalpas et al., 1998). On the other hand, the two main causes of liver disease (alcohol and virus) are partially preventable as they both depend on risk behaviors. Avoidance of alcohol intake is required to eliminate progressive liver disease in alcoholics. This is best achieved by using educational and social programs to convince patients and their caretakers of the great necessity to eliminate alcohol intake (Leevy and Moroianu, 2005).

The results of this study do not explain the influence of genotyping pattern in the evolution of the cause of alcoholic liver disease but indicate that in patients with hepatitis B infection alcohol is associated with a more severe liver disease, highlighted in older patients.

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References

Anderson, P. (1996a). Alcohol and primary health care. WHO Reg. Publ. Eur. Ser. *64*, 1–90.

Anderson, P. (1996b). Health, society and alcohol. Addiction *91*, 749–752.

Anttila, P., Jarvi, K., Latvala, J., Romppanen, J., Punnonen, K., and Niemela, O. (2005). Biomarkers of alcohol consumption in patients classified according to the degree of liver disease severity. Scand. J. Clin. Lab. Invest. *65*, 141–151.

Balasubramanian, S., and Kowdley, K. V. (2005). Effect of alcohol on viral hepatitis and other forms of liver dysfunction. Clin. Liver Dis. *9*, 83–101.

Basaras, M., Arrese, E., Blanco, S., Sota, M., de las Heras, B., and Cisterna, R. (2007). Characterization of hepatitis B virus genotypes in chronically infected patients. Rev. Esp. Quimioter. *20*, 442–445.

Bassendine, M. F., Della Seta, L., Salmeron, J., Thomas, H. C., and Sherlock, S. (1983). Incidence of hepatitis B virus infection in alcoholic liver disease, HBsAg negative chronic active liver disease and primary liver cell cancer in Britain. Liver. *3*, 65–70.

Cacoub, P., Saadoun, D., Bourliere, M., Khiri, H., Martineau, A., Benhamou, Y., et al. (2005). Hepatitis B virus genotypes and extrahepatic manifestations. J. Hepatol. *43*, 764–770.

Chung, N. S., Kwon, O. S., Park, C. H., Kim, Y. N., Cho, G. H., Lee, J. J., et al. (2007). A comparative cross-sectional study of the development of hepatocellular carcinoma in patients with liver cirrhosis caused by hepatitis B virus, alcohol, or combination of hepatitis B virus and alcohol. Korean J. Gastroenterol. *49*, 369–375.

Corrao, G., Torchio, P., Zambon, A., Ferrari, P., Arico, S., and di Orio, F. (1998). Exploring the combined action of lifetime alcohol intake and chronic hepatotropic virus infections on the risk of symptomatic liver cirrhosis. Collaborative groups for the study of liver diseases in Italy. Eur. J. Epidemiol. *14*, 447–456.

Dawson, D. A. (2000). Alcohol consumption, alcohol dependence, and all-cause mortality. Alcohol. Clin. Exp. Res. *24*, 72–81.

Donohue, T. M. Jr. (2007). Alcohol-induced steatosis in liver cells. World J. Gastroenterol. *13*, 4974–4978.

Echevarria, J. M., and Leon, P. (2004). Hepatitis B virus genotypes identified by a line probe assay (LiPA) among chronic carriers from Spain. Enferm. Infecc. Microbiol. Clin. *22*, 452–454.

Feld, J. J., and Liang, T. J. (2006). Hepatitis C—identifying patients with progressive liver injury. Hepatology. *43*, S194–S206.

Fiellin, D. A., Samet, J. H., and O'Connor, P. G. (1998). Reducing bias in observational research on alcohol withdrawal syndrome. Subst. Abus. *19*, 23–31.

Gao, B. (2002). Interaction of alcohol and hepatitis viral proteins: implication in synergistic effect of alcohol drinking and viral hepatitis on liver injury. Alcohol. *27*, 69–72.

Goudeau, A., Maupas, P., Dubois, F., Coursaget, P., and Bougnoux, P. (1981). Hepatitis B infection in alcoholic liver disease and primary hepatocellular carcinoma in France. Prog. Med. Virol. *27*, 26–34.

Gramenzi, A., Caputo, F., Biselli, M., Kuria, F., Loggi, E., Andreone, P., et al. (2006). Review article: alcoholic liver disease—pathophysiological aspects and risk factors. Aliment Pharmacol. Ther. *24*, 1151–1161.

Hoek, J. B., and Pastorino, J. G. (2004). Cellular signaling mechanisms in alcohol-induced liver damage. Semin. Liver Dis. *24*, 257–272.

Inoue, K. (1977). The clinicopathological features of the alcoholic liver injury in Japan and its etiological relationship to hepatitis B virus. Gastroenterol. Jpn. *12*, 230–240.

Kao, J. H. (2002). Hepatitis B viral genotypes: clinical relevance and molecular characteristics. J. Gastroenterol. Hepatol. *17*, 643–650.

Kondili, L. A., Tosti, M. E., Szklo, M., Costantino, A., Cotichini, R., Resuli, B., et al. (1998). The relationships of chronic hepatitis and cirrhosis to alcohol intake, hepatitis B and C, and delta virus infection: a case-control study in Albania. Epidemiol. Infect. *121*, 391–395.

Larkin, J., Clayton, M. M., Liu, J., and Feitelson, M. A. (2001). Chronic ethanol consumption stimulates hepatitis B virus gene expression and replication in transgenic mice. Hepatology. *34*, 792–797.

Leevy, C. M., and Moroianu, S. A. (2005). Nutritional aspects of alcoholic liver disease. Clin. Liver Dis. *9*, 67–81.

Livingston, M., and Room, R. (2009). Variations by age and sex in alcohol-related problematic behaviour per drinking volume and heavier drinking occasion. Drug Alcohol Depend. *101*, 169–175.

Mann, R. E., Smart, R. G., and Govoni, R. (2003). The epidemiology of alcoholic liver disease. Alcohol Res. Health. *27*, 209–219.

Marcellin, P., Pequignot, F., Delarocque-Astagneau, E., Zarski, J. P., Ganne, N., Hillon, P., et al. (2008). Mortality related to chronic hepatitis B and chronic hepatitis C in France: evidence for the role of HIV coinfection and alcohol consumption. J. Hepatol. *48*, 200–207.

Mills, P. R., Follett, E. A., Urquhart, G. E., Clements, G., Watkinson, G., and Macsween, R. N. (1981). Evidence for previous hepatitis B virus infection in alcoholic cirrhosis. Br. Med. J. (Clin. Res. Ed.) *282*, 437–438.

Morgan, M. Y., and Sherlock, S. (1977). Sex-related differences among 100 patients with alcoholic liver disease. Br. Med. J. *1*, 939–941.

Nalpas, B., Pol, S., Thepot, V., Zylberberg, H., Berthelot, P., and Brechot, C. (1998). ESBRA 1997 Award lecture: relationship between excessive alcohol drinking and viral infections. Alcohol Alcohol. *33*, 202–206.

Ohnishi, K., Iida, S., Iwama, S., Goto, N., Nomura, F., Takashi, M., et al. (1982). The effect of chronic habitual alcohol intake on the development of liver cirrhosis and hepatocellular carcinoma: relation to hepatitis B surface antigen carriage. Cancer. *49*, 672–677.

Oliveira, J., Parente, F., Sá, A., et al. (2001). Hepatocellular carcinoma: Description of cases from the Coimbra University Hospital. Rev. Gastroenterol. Cir. *18*, 112–121.

Oshima, A., Tsukuma, H., Hiyama, T., Fujimoto, I., Yamano, H., and Tanaka, M. (1984). Follow-up study of HBs Ag-positive blood donors with special reference to effect of drinking and smoking on development of liver cancer. Int. J. Cancer. *34*, 775–779.

Pereira, F. E., Goncalves, C. S., and Zago Mda, P. (1994). The effect of ethanol intake on the development of hepatocellular carcinoma in HBsAg carriers. Arq. Gastroenterol. *31*, 42–46.

Pinto, A. (2000). *Epidemiology of the problemas associated with alcohol consumption in Portugal*. Portugal: Permanyer. 9–26.

Pugh, R. N., Murray-Lyon, I. M., Dawson, J. L., Pietroni, M. C., and Williams, R. (1973). Transection of the oesophagus for bleeding oesophageal varices. Br. J. Surg. *60*, 646–649.

Royston, P., and Altman, D. G. (1994). Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling. Appl. Statist. *43(3)*, 429–467.

Sanchez-Tapias, J. M., Costa, J., Mas, A., Bruguera, M., and Rodes, J. (2002). Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in Western patients. Gastroenterology. *123*, 1848–1856.

Saunders, J. B., Davis, M., and Williams, R. (1981). Do women develop alcoholic liver disease more readily than men? Br. Med. J. (Clin. Res. Ed.) *282*, 1140–1143.

Saunders, J. B., Wodak, A. D., Morgan-Capner, P., White, Y. S., Portmann, B., Davis, M., et al. (1983). Importance of markers of hepatitis B virus in alcoholic liver disease. Br. Med. J. (Clin. Res. Ed.) *286*, 1851–1854.

Spirits, C. F. D. (2002). *World Drink Trends 2002. International Beverage Consumption and Production Trends*. Henley-on-Thames, UK: NTC Publications.

Stefos, A., Gatselis, N., Zachou, K., Rigopoulou, E., Hadjichristodoulou, C., and Dalekos, G. N. (2009). Descriptive epidemiology of chronic hepatitis B by using data from a hepatitis registry in Central Greece. Eur. J. Intern. Med. *20*, 35–43.

Villa, E., Rubbiani, L., Barchi, T., Ferretti, I., Grisendi, A., de Palma, M., et al. (1982). Susceptibility of chronic symptomless HBsAg carriers to ethanol-induced hepatic damage. Lancet. *2*, 1243–1244.

Wang, L. Y., You, S. L., Lu, S. N., Ho, H. C., Wu, M. H., Sun, C. A., et al. (2003). Risk of hepatocellular carcinoma and habits of alcohol drinking, betel quid chewing and cigarette smoking: a cohort of 2416 HBsAg-seropositive and 9421 HBsAg-seronegative male residents in Taiwan. Cancer Causes Control. *14*, 241–250.

Weisberg, I. S., Brown, R. S. Jr., and Sigal, S. H. (2007). Hepatitis B and end-stage liver disease. Clin. Liver Dis. *11*, 893–916.

WHO. Adult per capita consumption; 2008a.

WHO. W.H.O.S.I.S; 2008b.

WHO. Alcohol consumption and harm; 2009.