

CLINICAL STUDIES

Non-alcoholic fatty liver disease and its association with obesity, insulin resistance and increased serum levels of C-reactive protein in Hispanics

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Keywords

epidemiology – fatty liver – high-sensitivity C-reactive protein – Hispanics – insulin resistance – steatohepatitis

Abbreviations

ALT, alanine aminotransferase; ATP III, adult treatment panel III; BMI, body mass index; CI, confidence interval; DPC, diagnostics product corporation; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; ROC, receiver operating characteristic

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Non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of fat in the liver in the absence of alcohol consumption (>20 g/day) and other causes of chronic liver disease such as viral hepatitis or drugs (1, 2). This clinical and pathological entity encompasses a wide spectrum of liver damage ranging from simple steatosis to inflammation, fibrosis and cirrho-

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder of the liver, which may progress to fibrosis or cirrhosis. Recent studies have shown a significant impact of ethnicity on susceptibility to steatosis-related liver disease. **Aims:** To estimate the prevalence of NAFLD among Chilean Hispanics as well as the clinical and biochemical variables associated with the disease. **Methods:** Population-based study among Chilean Hispanics. The diagnosis of NAFLD was made on the basis of ultrasound evidence of fatty liver and absence of significant alcohol consumption and hepatitis C virus infection. **Results:** A total of 832 Hispanic subjects were included. Ultrasound findings revealed diffuse fatty liver in 23% of the subjects. Variables associated with fatty liver in multivariate analysis were body mass index > 26.9 [odds ratio (OR) 6.2; 95% confidence interval (CI) 3.3–11.5], abnormal aspartate aminotransferase levels (OR 14; 95% CI 8.2–23.7), presence of insulin resistance as measured by homeostasis model assessment-insulin resistance (OR 3; 95% CI 1.8–4.8) and serum levels of high-sensitivity C-reactive protein (hs-CRP) greater than 0.86 mg/L (OR 2.9; 95% CI 1.6–5.2). Among subjects with NAFLD, levels of hs-CRP were similar regardless of the alanine aminotransferase (ALT) level. **Conclusions:** Chilean Hispanics exhibit a high prevalence of NAFLD. Obesity, insulin resistance, abnormal aminotransferase levels and elevated hs-CRP were independently associated with the presence of NAFLD. ALT elevation underestimates the presence of ultrasonographical fatty liver, whereas hs-CRP is a sensitive independent marker of NAFLD, which may be useful for detecting fatty liver in the general population.

sis. More recently, NAFLD has also been associated with the development of hepatocellular carcinoma (3).

Available epidemiological data on NAFLD indicate that this disease is an increasingly common problem worldwide (4). However, marked variability in prevalence rates has been reported owing to different study designs and different patient recruitment

criteria (4, 5). Interestingly, recent reports showed important racial and gender variations in NAFLD, where Hispanics showed the highest prevalence of fatty liver or elevated aminotransferase levels compared with Caucasian and African Americans in the United States (6–8). This may reflect different genetic susceptibility to the metabolic syndrome.

Approximately 20–40% of patients with NAFLD have histological evidence of fibrosis, necrosis and inflammation (9, 10). These alterations define a more advanced form of NAFLD called non-alcoholic steatohepatitis (NASH), which is now considered the first cause of cryptogenic cirrhosis (11). Liver function tests, particularly serum aminotransferases, are often used as screening tests in asymptomatic patients. However, there is ample evidence that these tests lack sensitivity and specificity for liver disease and cirrhosis (12). Approximately 80% of individuals who have hepatic steatosis in the general population have serum levels of alanine aminotransferase (ALT) within the normal range, indicating that ALT is not a sensitive marker of this condition (7, 13). Other studies have linked NAFLD with components of the metabolic syndrome such as obesity, diabetes, hypertension and hyperlipidaemia (14, 15). In particular, advanced stages of NAFLD (i.e. NASH/fibrosis) have been independently associated with the presence of insulin resistance and hypertension (16).

High-sensitivity C-reactive protein (hs-CRP) is an acute-phase reactant and a non-specific marker of low-grade inflammation. It has been associated with conditions related to the metabolic syndrome and arteriosclerosis (17, 18). Serum levels of hs-CRP are usually elevated in obesity, dyslipidaemia and hyperglycaemia, all features of the metabolic syndrome (19). However, the relationship between hs-CRP and NAFLD is not well established (18–21).

The aim of this study was to estimate the prevalence of NAFLD in a Chilean Hispanic population, with a well-characterized and homogeneous genetic background, and to determine which clinical and biochemical variables are associated with this disease.

Patients and methods

Study design

This study is part of a large-scale epidemiological project that studied the prevalence and natural history of cholelithiasis, which started in 1993 in the south-eastern urban area of Santiago, Chile (22). A cluster random sampling methodology was used. Households from this area were randomly selected by a computer program, assuring a proportional representation of each neighbourhood and dwelling unit. Individuals

from houses or apartments of each selected block were invited to participate in 1993. Individuals were followed and data and blood samples were re-assessed in the year 2000 from those subjects who agreed to participate in the current study.

Subjects who accepted to participate in this study were interviewed after an informed consent was obtained. Inclusion criteria were: age ≥ 18 years old and Hispanic ethnicity, defined as having four surnames of Spanish origin and four generations living in Chile (20). Subjects with Amerindian ancestry were excluded based on a relatively objective estimation of the aborigine genetic pool present in the Hispanic populations, which was determined by calculating the Amerindian Admixture Index from the ABO blood group distribution, assuming a hybrid population of biparental origin (21, 22). The Amerindian maternal origin was defined by the determination of the mtDNA polymorphisms in a selected sample of 350 unrelated Hispanics. For this purpose, genomic DNA was isolated from each subject (23). The four Amerindian founder haplotypes and sequencing analysis of the hypervariable D-loop region were performed in DNA samples (24–26).

Individuals with alcohol consumption greater than or equal to 20 g of alcohol per day, or positive antibodies to HCV were excluded. Serum hs-CRP values > 10 mg/L were excluded under the assumption that they may represent a concomitant acute inflammatory illness (23). This study was approved by the Institutional Review Board for Human Studies of the Pontificia Universidad Católica de Chile and all participants gave their informed consent.

Study measurements

One-time evaluation included a personal interview and filling of a precoded questionnaire. Individual anthropometric measurements were recorded. Blood samples were obtained for biochemical tests, and a physician performed an abdominal ultrasound on each subject.

Interview

The screening protocol included a precoded questionnaire with socioeconomic data, medical history including previously known diagnosis of hypertension and diabetes, a detailed history of current alcohol consumption with an estimation of daily intake in grams per day and concomitant medication use.

Anthropometric measurements

Anthropometric measurements were performed by the interviewer, including weight, height, body mass index

(BMI) and waist-to-hip circumference. Obesity was defined as a BMI ≥ 30 kg/m².

Biochemical tests

Blood samples for each individual were obtained. Serum fasting glucose, serum ALT and serum lipid profile measurements were performed in an automatized Roche[®] Hitachi Modular chemistry analyzer (Hitachi, Tokyo, Japan). Hepatitis C virus (HCV) antibodies were detected by a third-generation immunoassay test, using the microparticle enzyme immunoassay technique on the Abbott AxSYM[®] (Abbott Park, IL, USA). Insulin serum level was measured with the Immulite 2000 equipment with DPC reactive (Diagnostics Product Corporation, Los Angeles, CA, USA). Insulin resistance was determined by the homeostasis model assessment-insulin resistance (HOMA-IR) method, which has a strong correlation with the clamp method to determine total glucose disposal and to assess insulin sensitivity (24). HOMA-IR was calculated according to the formula: insulin (μ U/ml) \times fasting plasma glucose (mmol/L)/22.5 (25). Insulin resistance was defined in Chile by Acosta *et al.* (27) according to the WHO criteria. HOMA-IR > 2.6 is considered to indicate insulin resistance in non-diabetic subjects in Chile. Metabolic syndrome, defined according to ATP-III criteria, was established when three or more of the following abnormalities were fulfilled: waist circumference > 102 cm in men and 88 cm in women; serum triglycerides level ≥ 150 mg/dl (1.69 mmol/L); HDL cholesterol concentration < 40 mg/dl (1.04 mmol/L) in men and < 50 mg/dl (1.29 mmol/L) in women; blood pressure $\geq 130/85$ mmHg; or serum glucose level ≥ 110 mg/dl (6.1 mmol/L) (26). Determination of serum hs-CRP was performed with a latex particle enhanced nephelometric immune assay, in BN ProSpec equipment, Dade Behring[®] (Deerfield, IL, USA). Abnormal aminotransferase levels were defined as ALT > 30 IU/L in men and ALT > 19 IU/L in women (27). Diabetes mellitus was defined using the American Diabetes Association criteria (28). Previously known hypertension was defined as blood pressure equal to or $> 140/90$ mmHg on two different occasions (29), a previously known diagnosis or antihypertensive drugs' use.

Radiological examinations

Abdominal ultrasounds were performed by two well-trained physicians using a 3.5 MHz linear transducer (Toosbee, Toshiba, Japan). Fatty liver was defined as liver parenchyma with echogenicity higher than the right kidney cortex on two different probe positions,

the presence of vascular blurring and deep attenuation (30). All images were recorded.

Statistical analysis

Data were age and sex adjusted when appropriate. χ^2 test and analysis of covariance were used to compare frequencies and continuous variables respectively. The receiver-operating characteristic (ROC) curve was used to obtain a cut-off value when the area under the curve was larger than 60% (31). Based on ROC curve cut-off values, continuous variables were transformed into discrete ones. Univariate and multivariate analyses were performed. Potential metabolic and biochemical variables associated with fatty liver were examined by comparing the means and proportions of variables among people with or without fatty liver. To study these relationships further, univariate logistic regression analysis was performed. In order to identify independent variables associated with NAFLD, a step-wise procedure for a multivariate logistic regression analysis was carried out, which included variables that appeared significant in univariate analysis. Highly correlated variables were excluded to avoid multicollinearity (i.e. insulin and HOMA-IR).

The χ^2 trend test was performed to evaluate possible statistical tendencies for the additive value of variables found to be significant in multivariate analysis. Analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA), R and S-PLUS (Insightful Corp, Seattle, WA, USA) softwares. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Differences were considered significant when *P*-values < 0.05 .

Results

Out of a target population of 1584 middle-low socioeconomic Hispanics, 959 patients agreed to participate in the current study. After exclusion criteria, a total of 832 Hispanic subjects (66.5% females, mean age 48.7 ± 13.2) were included in the analysis (Fig. 1). The general characteristics of the sample population study are shown in Table 1. A high prevalence of overweight subjects was found in this study population (mean BMI = 28.1 ± 5) and 23.2% of the population was considered obese. The prevalence of diabetes mellitus was 4.2% in this population. A mean HOMA-IR of 3.2 ± 3.2 was observed in our population. Subjects with NAFLD showed a higher frequency of insulin resistance (HOMA-IR > 2.6), with 72.3% compared with subjects without NAFLD (40.5%, *P*-value = 0.0001). A HOMA value of 2.16 or higher was the cut-off value associated with NAFLD in this study based on the ROC curve constructed for HOMA and ultrasonographical fatty liver.

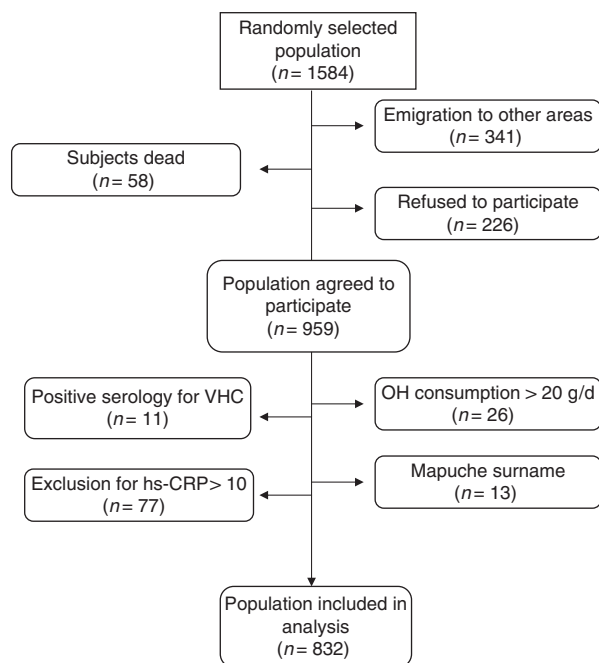


Fig. 1. Study design and flowchart.

Prevalence of non-alcoholic fatty liver disease

The prevalence of primary NAFLD, diagnosed by ultrasound, was 23.4%. Women exhibit a higher tendency of NAFLD than men, but no significant statistical gender difference was demonstrated. Individuals with fatty liver disease were older than subjects with normal ultrasound (50.2 ± 11.1 vs. 48.3 ± 13.7 years, respectively, $P < 0.001$). The highest prevalence of NAFLD was found in the fifth decade of life in both genders: 36.4% in women and 29% in men respectively.

Univariate analysis for fatty liver disease

Variables discretized according to cut-off levels obtained by ROC curves were BMI, ALT levels, HOMA-IR and hs-CRP. The optimal cut-off value for serum hs-CRP assessed by ROC curves was 0.86 mg/L, with a high sensitivity (88.4%) and a low specificity (34.8%). For ALT the optimal cut-off value, assessed by the ROC curve, was 14 IU/L with 100% sensitivity and 79% specificity. The results from univariate analysis of variables associated with fatty liver are shown in Tables 2 and 4.

Multivariate analysis

Variables independently associated with NAFLD in multivariate analyses were BMI (OR = 6.2, 95% CI 3.3–11.5), hs-CRP (OR = 2.9, 95% CI 1.6–5.2), ALT

Table 1. General characteristics of the Hispanic population

Variable	Hispanics
Subjects*	832
Sex (men/women)†	279 (33.5%)/553 (66.5%)
Age (year)†	48.7 ± 13.2
Fatty liver*	195 (23.4%)
BMI (kg/m ²)†	28.1 ± 5
Waist to hip ratio†	0.3 ± 1.2
Abdominal perimeter (cm)†	91.9 ± 44.3
Blood pressure > 130/85 mmHg†	328 (39.4%)
Serum glucose level (mg/dl)†	97.7 ± 35.6
Insulin (μU/ml)†	12.8 ± 9.4
HOMA-IR†	3.2 ± 3.2
Total cholesterol (mg/dl)†	211.2 ± 42.8
LDL cholesterol (mg/dl)†	133.4 ± 36.9
HDL cholesterol (mg/dl)†	49.9 ± 12.8
Triglycerides (mg/dl)†	138.2 ± 86.6
hs-CRP (mg/L)†	2.5 ± 2.4
Hypertension*, ‡	206 (24.8%)
Diabetes mellitus*	67 (8.1%)
Metabolic syndrome*	213 (25.6%)
ALT (IU/L)†	10.3 ± 7.1

*n (%).

†Mean (SD).

‡Patients with known hypertension or subjects with blood pressure $\geq 140/90$ mmHg on physical examination.

ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

level (OR = 13.95, 95% CI 8.2–23.7) and HOMA-IR (OR = 2.97, 95% CI (1.8–4.8) (Table 3).

The probability of finding hyperechogenic liver in ultrasound increased when variables, identified previously by multivariate analysis, coexisted. The results are shown in Figure 2. Because there were few variables, it was not possible to demonstrate the existence of an exponential or a quadratic tendency with a P (trend) $< 2.2 \times 10^{-16}$ and χ^2 208.98.

Discussion

Non-alcoholic fatty liver disease is increasingly being recognized as one of the most common causes of chronic liver disease worldwide. Thus, epidemiological information, such as that provided by the present study, is needed to design strategies for the prevention and treatment of the conditions. Although the definition of NAFLD is histological, it is impractical to use such a definition in epidemiological studies. Surrogate markers of NAFLD have been used, including abnormal ALT level and imaging studies of the liver (i.e. hyperechogenic liver assessed by ultrasound), leading to the concept of 'presumed NAFLD' when heavy alcohol consumption and other causes of liver injury

Table 2. Univariate analysis of variables associated with ultrasonographical fatty liver in the Hispanic population

Variables	Fatty liver at ultrasound	Normal liver at ultrasound	P-value	OR	95% CI for OR
Subjects (<i>n</i> = 832)	<i>n</i> = 195	<i>n</i> = 637			
Sex (men/women)†	69 (35.4%)/126 (64.6%)	209 (32.8%)/428 (67.2%)	0.56	1.1	0.79–1.55
Age (year)‡	50.2 ± 11.1	48.3 ± 13.7	0.01*	1.01	0.1–1.03
Waist to hip ratio‡	0.4 ± 0.1	0.3 ± 0.14	< 0.0001*	5.5	3.88–7.79
Abdominal perimeter (cm)‡	107.4 ± 87.6	87.2 ± 12	< 0.0001*	1.08	1.07–1.1
Glucose (mg/dl)	111.1 ± 51.1	93.7 ± 28.2	< 0.0001*	1.01	1.01–1.02
Insulin (μU/ml)‡	15.7 ± 7.4	11.9 ± 9.7	< 0.0001*	1.05	1.03–1.07
Total cholesterol (mg/dl)‡	220.1 ± 44.2	208.5 ± 42.1	0.0008*	1.01	1–1.01
HDL cholesterol (mg/dl)‡	45.6 ± 9.8	51.2 ± 13.4	< 0.0001*	0.96	0.94–0.97
LDL cholesterol (mg/dl)‡	139.1 ± 40.4	131.6 ± 35.6	0.01*	1.01	1–1.01
Triglycerides (mg/dl)‡	171.9 ± 88	127.9 ± 83.6	< 0.0001*	1.01	1–1.01
Hypertension†,§	70 (36.1%)	128 (20.1%)	< 0.0001*	2.23	1.57–3.16
Diabetes mellitus†	33 (16.9%)	34 (5.3%)	< 0.0001*	3.64	2.19–6.04
Metabolic syndrome†	95 (48.7%)	118 (18.5%)	< 0.0001*	4.31	3.05–6.09
BMI > 26.9 (kg/m ²)†	173 (89.7%)	291 (45.7%)	< 0.0001*	9.84	5.68–17.04
hs-CRP > 0.86 (mg/L)†	175 (89.7%)	417 (65.5%)	< 0.0001*	4.96	3–8.18
ALT > 14 (IU/L)†	85 (43.6%)	29 (4.6%)	< 0.0001*	16.54	10.36–26.4
HOMA-IR > 2.16†	166 (85.1%)	323 (50.7%)	< 0.0001*	5.57	3.64–8.5

*Considered statistically significant at $P < 0.05$.

†*n* (%).

‡Mean (SD).

§Patients with known hypertension or subjects with blood pressure $\geq 140/90$ mmHg on physical examination.

ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; HOMA-IR, homoeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

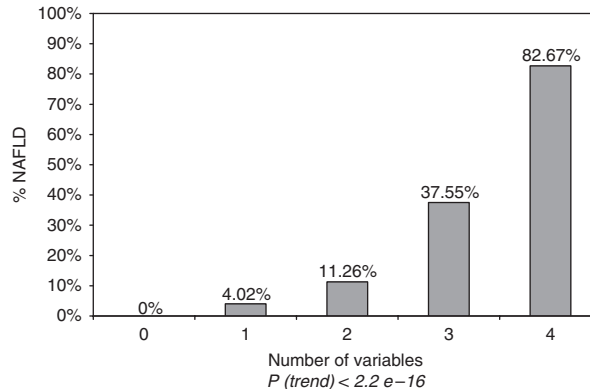
Table 3. Variables independently associated with the presence of ultrasonographical fatty liver in the multivariate analysis in the Hispanic population

Variable	OR	95% CI
BMI > 26.9 kg/m ² *	6.2	(3.34–11.51)
hs-CRP > 0.86 mg/L*	2.9	(1.62–5.19)
ALT > 14 IU/L*	13.95	(8.22–23.67)
HOMA-IR > 2.16*	2.97	(1.82–4.84)

*Cut-off values were obtained from ROC curves.

ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; HOMA-IR, homoeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; ROC, receiver-operating characteristic.

are excluded (28). The ultrasound sensitivity for histological steatosis ranges from 60 to 100% and is most often between 82 and 94% in several studies. The specificity is between 66 and 100%, with most studies reporting 82% or above (29). It must be considered that, ultrasound, and other imaging techniques such as computed tomography and magnetic resonance imaging, are insensitive to assess the different degrees of steatosis and also to detect fatty liver with < 25–30% of hepatocytes affected (30). This limitation must be considered in every population

**Fig. 2.** Probability of having hyperechogenic liver at ultrasound according to the number of variables significantly associated in the multivariate analysis.

study, as in the present one, based on non-invasive surrogate markers of NAFLD.

The actual prevalence of NAFLD in the general population is unknown. Estimations ranging from 3 to 23%, depending on the case definition used, whether alcohol consumption or viral hepatitis, were rigorously ruled out and the ethnic background of the population was studied (31–35). In studies that use ALT levels as a surrogate marker of NAFLD, prevalence

Table 4. Univariate analysis for Hispanic population with ultrasonographical fatty liver according to ALT level

Variables	Ultrasonographical fatty liver and normal aminotransferases levels	Ultrasonographical fatty liver with abnormal aminotransferases levels†	P-value	OR	95% CI for OR
Hispanics (n = 195)	n = 164	n = 31			
Sex (men/women)‡	63 (38.4%)/101 (61.6%)	6 (19.4%)/25 (80.7%)	0.048*	0.39	0.15–1
Age (year)§	50.6 ± 10.7	48.1 ± 12.8	0.24	0.98	0.95–1.02
Waist to hip ratio§	0.4 ± 0.1	0.4 ± 0.1	0.82	1.1	0.48–2.56
Abdominal perimeter (cm)§	103.8 ± 69.1	126.5 ± 152.3	0.23	1	1–1.01
Glucose (mg/dl)§	108.6 ± 47.2	123.9 ± 67.5	0.13	1.01	1–1.01
Insulin (μU/ml)§	15.6 ± 7.4	16.5 ± 7.6	0.49	1.02	0.97–1.07
Total cholesterol (mg/dl)§	219.9 ± 43.2	220.9 ± 49.5	0.94	1	0.99–1.01
HDL cholesterol (mg/dl)§	45.2 ± 9.7	47.3 ± 10.3	0.28	1.02	0.98–1.06
LDL cholesterol (mg/dl)§	140 ± 40.1	134.6 ± 42	0.46	1	0.99–1.01
Triglycerides (mg/dl)§	173.7 ± 86.4	162.6 ± 97	0.53	1	0.99–1
Hypertension‡,¶	58 (35.6%)	12 (38.7%)	0.72	1.16	0.52–2.54
Diabetes mellitus‡	28 (17.2%)	5 (16.1%)	0.9	0.93	0.33–2.64
Metabolic syndrome‡	83 (50.9%)	12 (38.7%)	0.29	0.58	0.21–1.6
BMI (kg/m ²)‡	31.5 ± 4.6	32.2 ± 4.7	0.47	1.03	0.95–1.12
hs-CRP (mg/L)‡	3.5 ± 2.5	4.5 ± 2.7	0.06	1.15	1–1.33
HOMA-IR‡	4.2 ± 2.7	5.1 ± 3.7	0.1	1.11	0.98–1.24

*Considered statistically significant at $P < 0.05$.

†Defined as ALT >30 IU/L in men and ALT >19 IU/L in women.

‡n (%).

§Mean ± SD.

¶Patients with known hypertension or subjects with blood pressure ≥ 140/90 mmHg on physical examination.

ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; HOMA-IR, homoeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

varies in part owing to the cut-off value used. For example, when using ALT higher than 43 IU/L, presumed NAFLD the prevalence is 2.8%; however, when using a lower cut-off level, the prevalence is around 13% (6, 12). Studies that use ultrasound usually show a higher prevalence of NAFLD than those that use the ALT level. In the Japanese general population, the prevalence of NAFLD was 19%, but when heavy alcohol drinkers were excluded, the prevalence decreased to 12.8% (33). Other studies have found a higher prevalence of NAFLD as has been reported by Singh *et al.* (36) in eastern India (24.5%) and Bellen-tani in the Dionysos Project, a cross-sectional study conducted in Italy that found a prevalence of 20% (34). The present study shows a prevalence of presumed NAFLD, assessed by ultrasound, of 23% in Chilean Hispanics, which is remarkably similar to the above-mentioned figures. It must be considered that subjects who drank more than 20 g/d of alcohol and those with chronic hepatitis C were excluded. Serology for hepatitis B virus infection was not determined because it does not represent a cause of hyperecho-genic liver and the estimated prevalence of this infection in Chile is very low (0.25%) (37). Indeed, the high prevalence of obesity in Chile influences the frequency

of NAFLD in our population. Recent epidemiological data from our country indicate that the frequency of overweight/obesity in the general population is significant in Chile. [37.8% of overweight subjects (BMI 25–30) and 23.2% of Obesity (BMI > 30) in a National Survey conducted in 2003 (38)]. This may be related to the Hispanic background of our population. Data in this regard indicate that a higher prevalence of obesity is seen in similar populations such as Mexican Americans when compared with non-Hispanic whites (7, 39, 40). Moreover, studies based on more sensitive methods, such as measurement of hepatic triglyceride content using proton magnetic resonance spectroscopy, have reported that the prevalence of liver steatosis shows a dramatic variation with ethnicity, being clearly higher in Hispanics than in Whites and African-American subjects (7).

The high prevalence of NAFLD in the Chilean population might be related to cirrhosis mortality rate. The age-adjusted mortality rate for cirrhosis in Chile was 32 per 100 000 habitants in 1998 (41) this figure being among the highest in the world. For comparison, the mortality rate from cirrhosis in the United States was 9.3 per 100 000 habitants (42). It is important to note that Chile has a lower prevalence of hepatitis C

infection than in the United States (1.15 vs. 1.8%) and similar per capita alcohol consumption (8.34 vs. 9.47 kg/year per person) (43–45). Taken together, these data suggest that there might be an increased susceptibility to liver disease in Chileans that is not explained by viral hepatitis C or alcohol consumption. Rather, NAFLD could represent a relevant co-factor for cirrhosis regardless of the cause of liver disease.

As mentioned before, this might be related to the Hispanic background of our population. Of note, Hispanic ethnicity seems to be related to insulin resistance (6), NAFLD (7) and cirrhosis (46, 47) and mortality owing to cirrhosis in US Hispanics has been reported to be 1.6 greater than the mortality rate observed for the non-Hispanic white population in 2005 (42). Because most North and South American Hispanic population harbour a significant degree of native American (Amerindian) genetic admixture (20, 48), it can be hypothesized that Amerindian genes predispose to NAFLD. This is consistent with studies showing increased genetic predisposition to diabetes and gallstones in native Americans, as has been shown in Pima and Mapuche Indians (12, 20, 49).

Several studies have suggested the association of NAFLD with obesity, diabetes, hypertension and hyperlipidaemia, features of the metabolic syndrome (14, 50, 51). The present report confirms these previous observations, showing a strong independent association of obesity and insulin resistance assessed by HOMA-IR with NAFLD in this Hispanic population.

Alanine aminotransferase level showed the strongest association with fatty liver, a finding that is not surprising because this is a commonly used method to detect NAFLD in the general population (6). The specificity of elevated ALT level for presumed NAFLD was very high (99%). However, the sensitivity of abnormal ALT level using cut-off values of ALT > 30 IU/L in men and ALT > 19 IU/L in women was poor (15.9%), underestimating the prevalence of NAFLD (52). Aminotransferase levels are commonly used in clinical practice to estimate liver inflammation, but the use of ALT level as a reliable indicator of the severity of NAFLD is controversial as aminotransferase levels may fluctuate with normal levels in more than two-thirds of NASH patients at any given time (53). We found that among subjects with fatty liver at ultrasound, those with normal and abnormal ALT levels were very similar, providing indirect evidence that ALT is not sorting out different conditions or stages of the disease.

A remarkable finding of the current study is the strong independent association of serum hs-CRP with presumed NAFLD. This association had an OR similar to HOMA-IR in the multivariate analysis. The optimal

cut-off value assessed by the ROC curve was 0.86 mg/L, with a high sensitivity (88.4%) and a low specificity (34.8%). Previous reports have shown controversial results regarding this association (48–51). Some studies failed to show an association of hs-CRP with the histological severity of NAFLD (9). In contrast, there are several case and control studies suggesting an association between elevated serum levels of CRP and the presence of NAFLD (48, 54). Recently, a study reported increased serum levels of hs-CRP in cases of histologically confirmed NASH compared with simple non-progressive steatosis (21). Our results indicate that hs-CRP may be considered as another non-invasive marker of NAFLD, adding a new tool to the repertoire of the primary care physicians or hepatologists attempting to establish a diagnosis of NAFLD.

In conclusion, this study demonstrates a high prevalence of NAFLD among Chilean Hispanics, showing at the same time that obesity, insulin resistance and ALT level are independently associated with NAFLD in this population. The high prevalence of NAFLD may account for the high mortality rates for cirrhosis seen in our country. On the other hand, the strong association of serum hs-CRP and NAFLD leads to new questions about the pathogenesis of the disease and also hints that hs-CRP measurement may be useful to select patients with NAFLD among subjects with a normal ALT level. In the future, a follow-up of this cohort will provide information about the predictive value of serum hs-CRP for NAFLD and clinically relevant outcomes such as development of cirrhosis, portal hypertension, hepatocellular carcinoma and liver-related mortality.

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