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Predictors of Nonalcoholic Steatohepatitis (NASH) in Obese Patients Undergoing Gastric Bypass

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Background: Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are conditions gaining increasing recognition in hepatology as a potential cause of cirrhosis and end-stage liver disease. Obesity is one of the main risk factors. The aims of this study were to determine the frequency of NAFLD in obese patients and to identify variables that predict NASH.

Methods: A prospective study was conducted of obese patients undergoing gastric bypass over a 20-month period. Assessment included liver function tests and evaluation of insulin resistance with the homeostatic model assessment (HOMA-IR). Liver biopsy was performed in all patients at the time of surgery. Clinical and biochemical variables were analyzed using a multivariate analysis to identify independent predictors of NASH.

Results: 127 consecutive patients were included (62% female, 38% male, mean age 40±11 years, mean body mass index 42±6 kg/m^2). Arterial hypertension was present in 52 patients (41%) and type 2 diabetes in 18 (14%). NAFLD was confirmed in 80 patients (63%), 47 (37%) had simple steatosis, and 33 (26%) had NASH. Cirrhosis was found in 2 patients corresponding to 1.6% of the total population. On multivariate analysis, AST >31 (IU/L) (OR 3.38, CI 1.17-9.8) and HOMA-IR >5.8 (OR 4.18, CI 1.39-12.49) independently predicted NASH.

Conclusions: NAFLD is highly prevalent in morbidly obese patients. A high proportion of these patients exhibit NASH on histological examination. Insulin resistance represents the main predictor of NASH.

Key words: Morbid obesity, steatohepatitis, liver, insulin resistance, metabolic syndrome

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver disorders today.[1] Prevalence in the general population has reached epidemic proportions in Western countries,[2] ranging from 16 to 20% and rising to 90% in obese patients.[3] Although NAFLD is considered a benign and non-progressive disease, a subgroup of patients can show inflammatory changes and fibrosis, a condition known as nonalcoholic steatohepatitis (NASH).[4] In these patients, 7 to 32%[5,6] can develop more advanced forms of hepatic damage including advanced fibrosis,[6] cirrhosis[7] and hepatocelular carcinoma.[8]

Obesity and insulin resistance are strongly associated with NASH,[9] with a reported prevalence of 2.5% to 70% in obese patients undergoing bariatric surgery.[9,10] A variety of factors have been associated with NASH including type 2 diabetes, arterial hypertension, insulin resistance and abnormal aminotransferase levels on liver tests.[9,11] To identify factors that predict NASH can mandate patients for liver biopsy and close follow-up. The aim of this study was to determine the prevalence of NAFLD and define risk factors that predict NASH in liver histology.

Methods

This prospective study included 127 obese patients undergoing gastric bypass from April 2001 to

November 2002 in the Department of Digestive Surgery, Hospital Clínico Universidad Católica de Chile. All patients signed an informed consent before surgery.

We excluded patients with known alcohol consumption >20 g per day, patients with a mean corpuscular volume of red cells >100 fl, and those with chronic hepatic disease of a known origin (chronic viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, hemochromatosis, Wilson's disease, α-1 antitrypsin-deficiency-associated liver disease).

Preoperative evaluation included medical history, physical examination, nutritional cardiopulmonary and psychiatric evaluation. All patients met the criteria for obesity surgery with a body mass index (BMI) ≥40 kg/m^2 or ≥35 kg/m^2 with significant co-morbid conditions such as arterial hypertension, type 2 diabetes, sleep apnea or dyslipidemia.[12] Laboratory included complete blood tests and liver function tests: alanine (ALT) and aspartate (AST) aminotransferases, alkaline phosphatase (AP), γ-glutamyltranspeptidase (GGT), total bilirubin, albumin, prothrombin time (PT), fasting glucose, fasting insulin, total cholesterol, LDL, HDL, fasting triglyceride. Insulin resistance (IR) was evaluated using the homeostatic model assessment (HOMA-IR) described by Matthews.[13]

An intraoperative fine-needle liver biopsy was obtained at the beginning of the operation from the left lobe. An additional core was taken in case the sample was <10 mm in length. Liver biopsies were examined by a single pathologist (I.D.) who was unaware of the clinical and laboratory data, using hematoxylin-eosin stain and Masson's trichrome stain. Specimens were analyzed according to the American Association for the Study of the Liver Diseases Single Topic Conference,[14] considering Class I as simple steatosis, Class II as steatosis with lobular inflammation, Class III as additional presence of ballooned hepatocytes, and Class IV as the presence of either Mallory's hyaline or zone 3 fibrosis. Class III and IV were defined as NASH. These patients were further evaluated for grading and staging of NASH (*Grade 1* mild: steatosis in 33% to 66% of lobules, occasional ballooning in zone 3, mild lobular inflammation with or without mild portal inflammation; *Grade 2* moderate: steatosis, ballooning in zone 3, lobular inflammation noted with or without pericellular fibrosis, mild chronic inflammation, none or mild to moderate portal inflammation; *Grade 3* severe: steatosis usually >66%, marked ballooning especially zone 3, scattered acute and chronic inflamation, perisinusoidal fibrosis, mild or moderate portal inflammation, not predominant or marked. Fibrosis was classed as *Stage 1*: Zone 3 perivenular or pericellular fibrosis; *Stage 2:* as for stage 1 plus focal or extensive portal fibrosis; *Stage 3:* bridging fibrosis, focal or extensive; *Stage 4:* cirrhosis with or without residual perisinusoidal fibrosis).[14]

Comparison of clinical and biochemical variables between patients with normal histology, simple steatosis and NASH was performed using the Kruskal Wallis non-parametric test with Bonferroni test to identify the differences between groups and and chi-square for categorical data. Results are expressed as median (interquartile range), mean (standard deviation) or number of patients (percentage). Metabolic syndrome was defined according to the ATP III criteria[15] for patients having three or more of the following criteria: 1) Waist circumference >102 cm in men and >88 cm in women; 2) Hypertriglyceridemia ≥150 mg/dL; 3) High density lipoprotein cholesterol <40 mg/dL; 4) High blood pressure ≥130/ 85 mmHg; 5) High fasting glucose ≥110 mg/dL.

The independent effect of significant variables on NASH was assessed with a univariate and multivariate analysis using the logistic regression model by a stepwise procedure. Variables were considered statistically significant with $P<0.05$. Receiver operating curves (ROC) were constructed to identify the best cut-off point for AST and HOMA to include them in the multivariate analysis. This analysis was performed using the SAS 6.12 statistical software.

Results

Preoperative BMI of the 127 patients (79 female, 62%, 48 males 38%; mean age 40±11 years) was 42±6 kg/m^2 (mean ± SD). Of the patients, 52 (41%) had arterial hypertension and 18 (14%) type 2 diabetes. Clinical and biochemical parameters are shown in Table 1. Twelve patients (9.5%) had a BMI >50.

All liver biopsies were adequate for analysis. Normal histology was observed in 47 patients (37%) and NAFLD in 80 (63%). Histologic features

Table 1. Clinical and laboratory data of obese patients according to histological classification

Variable	Total	Normal (Class 0)	Steatosis (Classes 1 and 2)	NASH (Classes 3 and 4)	*P*-value
n (%)	127 (100)	47 (37)	47 (37)	33 (26)	
Age – yrs§	40±11	37±12[a]	41±9 b	42±12[b]	0.045
Male sex‡	48(38)	12(26)	17(36)	19(58)	0.014
BMI – kg/m^2§	42±6	41±5	43±7	43±5	0.125
Arterial hypertension‡	52 (41)	14(30)	20(42)	18(54)	0.097
Type 2 diabetes‡	18 (14)	1(2)	7(15)	10(30)	0.002
Metabolic syndrome‡	58 (45.6)	15(31.9)	22(46.8)	21(63.6)	0.01
ALT (IU/L)†	46±23	23±18[a]	24±17[a]	38±22[b]	<0.001
AST (IU/L)†	27±18	28±19[a]	48±20[ab]	67±43[b]	0.002
Alkaline phosphatase (IU/L)†	94±75	90±74	96±71	102±83	0.926
GGT (IU/L)†	29±14	22±14[a]	24±11[a]	48±21[b]	0.034
Total bilirubin (mg/dL)†	0.5±0.3	0.5±0.3[a]	0.4±0.3[a]	0.6±0.5[b]	0.023
Total cholesterol (mg/dL)†	212±179	208±178	218±176	205±183	0.583
LDL cholesterol (mg/dL)†	126±105	134±103	124±101	128±114	0.861
HDL cholesterol (mg/dL)†	45±38	49±43[a]	45±39[ab]	41±36[b]	0.027
Triglycerides (mg/dL)†	151±106	127±95[a]	167±118[b]	140±102[b]	0.038
Glucose (mg/dL)†	96±88	92±87	99±89	99±92	0.058
Insulin (µU/mL)†	21±16.	18±14[a]	21±14[ab]	28±19[b]	<0.001
Insulin resistance (HOMA-IR)†	5.2±3.6	4.6±3.0[a]	6.3±2.9[ab]	8.0±4.9[b]	0.001

BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyltranspeptidase.
§: Mean (standard deviation), *P*-value Kruskal-Wallis.
†: Median (interquartile range), *P*-value Kruskal-Wallis.
‡: n (%), *P*-value chi-square.
a, ab, b: When groups share the same letter, no significant difference by Bonferroni test.

of NASH were found in 33 patients (26%), Table 1. Grading and staging revealed as mild NASH 22 patients, moderate 5 and severe 6. Most patients (20 cases) had stage 1 fibrosis (Zone 3) and two had established cirrhosis (stage 4), Table 2.

Significant differences were found between patients with normal biopsy, simple steatosis and NASH. Nineteen patients (58%) were male in the NASH group, compared to 12 (26%) in the normal histology group ($P<0.05$). Moreover, 30% of the patients with NASH had diabetes (n=10) compared with 15% in the ones with simple steatosis and 26% in the group with normal biopsy (n=12). Significant differences were also observed in median AST, ALT, bilirubin, GGT, HDL cholesterol and triglycerides. Insulin resistance was significantly higher in patients with NASH (median HOMA-IR 8.0) than in patients with steatosis (median HOMA-IR 6.3) and normal histology (median HOMA-IR 4.6). Differences were also found in the proportion of patients who met the criteria for metabolic syndrome (31.9% in the normal histology group, 46.8% in simple steatosis and 63.6% in the NASH group) (Table 1).

Univariate analysis showed several factors with significant association with NASH, as type 2 diabetes (OR 4.6, CI 1.66-13.1), AST (OR 1.03, CI 1.01-1.04), ALT (OR 1.02, CI 1.01-1.05), GGT (OR 1.02, CI 1.00-1.03), HDL (OR 1.02, CI 1.00-1.03), Insulin (OR 1.05, CI 1.02-1.09) and HOMA-IR (OR 1.20, CI 1.05-1.37) (Table 3). Receiver operating curves were constructed to identify the

Table 2. Grading and fibrosis staging for NASH based on the American Association for the Study of the Liver Diseases single topic conference (n=33)[14]

Grading for NASH	n (%)
Grade 1, Mild	22 (66.6)
Grade 2, Moderate	5 (15.2)
Grade 3, Severe	6 (18.2)
Fibrosis staging for NASH *	
Stage 1	20 (60)
Stage 2	6 (18)
Stage 3	2 (6)
Stage 4	2 (6)

*3 patients with diagnosis of NASH had no zone 3 fibrosis in liver biopsy.

Table 3. Univariate and multivariate analysis of factors associated with NASH in obese patients

Univariate analysis	*P*-Value	Odds Ratio (OR)	Confidence Interval (CI)
Age (years)	0.18		
Female (yes/no)	0.008	0.3	0.15-0.74
BMI (kg/m^2)	0.48		
Weight (kg)	0.139		
Height	(m)	0.104	
Arterial hypertension (yes/no)	0.074	2.08	0.93-4.65
Type 2 diabetes (yes/no)	0.003	4.67	1.65-13.18
Sleep apnea (yes/no)	0.13		
AST (IU/L)	0.002	1.03	1.01-1.04
ALT (IU/L)	0.006	1.02	1.01-1.04
Alkaline phosphatase (IU/L)	0.72		
Total bilirubin (mg/dL)	0.01	9.08	1.61-51.1
GGT (IU/L)	0.036	1.02	1.00-1.03
Albumin (g/dL)	0.42		
Total cholesterol (mg/dL)	0.98		
LDL cholesterol (mg/dL)	0.58		
HDL cholesterol (mg/dL)	0.01	0.95	0.91-0.987
Triglycerides (mg/dL)	0.49		
Insulin resistance (HOMA-IR)	0.007	1.20	1.05-1.37
Insulin (μU/mL)	0.002	1.05	1.02-1.09
Glucose (mg/dL)		0.23	0.14-1.01
Multivariate analysis		**Odds Ratio (OR)**	**Confidence Interval (CI)**
HOMA >5.8	0.01	4.18	1.39-12.49
AST >31 (IU/L)	0.025	3.38	1.17-9.80

BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyltranspeptidase.

best cutt-off points for significant continuous variables. Patients with HOMA-IR <5.8 and AST <31 IU/L had 7.8% NASH on histology, whereas patients with both tests over the cut-off point had 50% NASH in their biopsy. Moreover, if these patients also had the diagnosis of type 2 diabetes, NASH was present in 75% of them (Figure 1). Multivariate analysis confirmed HOMA-IR >5.8 (OR 4.18, CI 1.39-12.49) and ALT >31 IU/L mg/dl (OR 3.38, CI 1.17-9.80) as the only independent predictors of NASH.

Discussion

NASH is a growing problem in clinical practice, with >50% of the U.S. population overweight and >20% obese.[16] In Chile and other developing countries, the situation is similar with a prevalence of obesity among women as high as 39%.[17] Europe has similar increasing obesity, especially in children.[18] With more patients undergoing bariatric surgery,[19] it is important to understand risk factors that could predict NAFLD and NASH.[20] In bariatric patients,

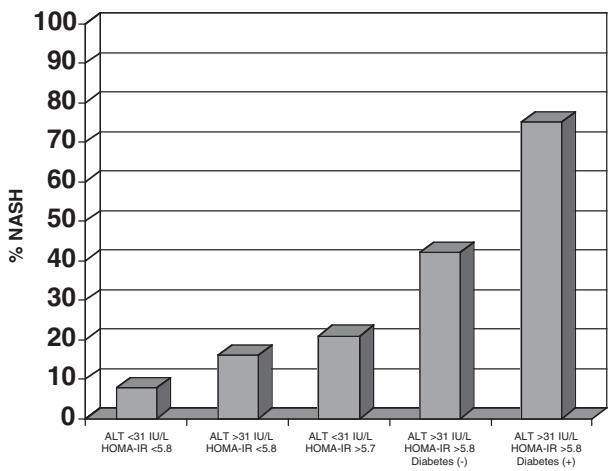

Figure 1. Association between NASH (%), abnormal AST level, HOMA-IR and presence of type 2 diabetes.

the prevalence can be as high as 90% for NAFLD and 70% for NASH.[9,21] In our population undergoing gastric bypass, the median BMI was 42 and the prevalence of NAFLD was 63%, less than in other series. A recent study reported 85% of the patients having NAFLD, with a median BMI of 57.[11] However, we did not find a significant association between BMI and histological changes.

NASH can affect a variable proportion of patients with NAFLD, depending on their risk factors. However, criteria for this diagnosis have not been uniform. With the recent classification proposed by the American Association for the Study of the Liver Diseases,[22] we made the diagnosis of NASH in 25% of the patients. This percentage is in accordance with similar studies in this population. Dixon et al[9] reported a series of 105 patients undergoing bariatric surgery with a 25% prevalence of NASH. Another recent study reported 33%.[11]

We identified significant differences between patients with simple steatosis, NASH and a normal biopsy, which may identify patients who need closer follow-up after surgery.[23] There were also some differences between patients with normal hystology and simple steatosis in the values of HOMA-IR and AST, suggesting that steatosis is the intermediate phase before necroinflammatory changes develop. Interestingly, we found significant differences in the three groups (normal, steatosis, NASH) in key elements of the metabolic syndrome, as others have noted.[24] In the normal histology group, 31.9% met the criteria for metabolic syndrome, compared to 46.8% in the ones with simple steatosis and 63.6% in the NASH group. This study gives further evidence that NASH should be considered as part of the metabolic syndrome.

Significant factors associated with NASH were identified: as in other studies, type 2 diabetes, abnormal aminotransferase levels, low HDL and especially serum fasting plasma insulin and insulin resistance expressed as HOMA-IR.[19] A recent study in 48 patients found only type 2 diabetes as the independent factor for NASH; however, they did not evaluate insulin resistance.[11] In our study, HOMA-IR was abnormal in 90% of patients (>2.6). Using ROC curves, we identified the best cut-off point to be 5.8 for HOMA-IR and ALT >31 IU/L. These were the only independent predictors of NASH. Similarly, Dixon et al[9] reported that HOMA-IR, ALT and arterial hypertension were independent predictors. They found a cut-off point of 5.0 for HOMA-IR and >40 IU/L for ALT. These results support the evidence that insulin resistance is an important element of NASH.[14,22]

Another interesting finding was the elevation of AST as predictive of NASH, with patients with HOMA-IR >5.8 and AST >31 IU/L having 2 times the incidence of NASH. This percentage rose to 75% if the patients were also diabetic. On the other hand, only 7.8% of the patients had NASH when they had normal AST and HOMA <5.8, suggesting a subgroup where intra-operative liver biopsy may not be mandatory.[25] Fibrosis in zone 3 was present in most patients with NASH (30/33), 90%. However, only 4 (3.1%) had advanced fibrosis (bridging fibrosis and cirrhosis). Other reports have found higher proportions of advanced fibrosis, up to 12%.[9,11] Two asymptomatic and non-diagnosed cases of cirrhosis (1.6%) were confirmed. Both patients had metabolic syndrome and high levels of insulin resistance (HOMA-IR of 10 and 20, respectively).

Having ruled out other etiologies, it is evident that the cause of cirrhosis could be the natural history of some patients with NAFLD. This observation has been reported where a high percentage of patients with cryptogenic cirrhosis had features of NASH and metabolic syndrome.[26] Furthermore, a recent report found hypertriglyceridemia and type 2 diabetes as factors in cirrhotic patients with hepatocelular carcinoma, suggesting also this risk for cirrhosis of NASH etiology.[8]

The natural history of NASH is still unclear.[27] In patients with NASH and a control biopsy, 32% were reported to have histological progression, defined as increased inflammation or fibrosis.[7] Therefore, the treatment of NASH should be one of the aims of bariatric surgery. A recent study confirmed a positive effect of weight loss after gastric banding on the key features of NASH and NAFLD – steatosis, necroinflamatory activity and fibrosis.[28] This improvement was even better in patients with metabolic syndrome. NASH resolved or remited in 82% of the patients. However, this effect has not been validated in patients undergoing gastric bypass, where a faster weight loss than after gastric banding could have a different effect, as other studies that have revealed increased inflammatory activity in the liver due to excess free fatty acids.[3]

In conclusion, this study confirms the high preva-

lence of asymptomatic liver disease in morbidly obese patients undergoing gastric bypass (NAFLD and NASH). It also indicates the strong relation of NASH to insulin resistance and the features of the metabolic syndrome. The proposed classification for NASH is a useful instrument for a systematic analysis of different series.

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