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Original/*Síndrome metabólico*

Predictive factors of non-alcoholic steatohepatitis: relationship with metabolic syndrome

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has been proposed as the hepatic manifestation of the metabolic syndrom (Ms), with insulin resistance (IR) as the common pathophysiological mechanism.

***Methods:* we included 145 patients with NAFLD proven liver biopsy. NAS-score was employed to grading NAFLD. We determined anthropometric measurements, basal blood pression (BP), biochemical measurements including high lipoprotein cholesterol (HDL-Chol), low-density lipoprotein cholesterol (LDL-Chol), triglycerides and leptin levels, homeostasis model assessment index (HOMA–IR), and abdominal ultrasound scan (US) was performed. Diagnosis of Ms was performed based on ATP III criteria.**

***Results:* average age was 43.6 + 11.2 years old and the mean body mass index (BMI) was 39 ± 10.7 kg/m². Sex distribution was: females 66 and males 79. Forty patients (27.5%) presented a NAS score > = 5. Waist circumference (p = 0.007), systolic and diastolic BP (p = 0.002 and p = 0.003 respectively), (HOMA-IR) (p<0.0001), body mass index BMI (p = 0.04), Ms (p = 0.04) and US-NAFLD were significantly associated with NAS-score > = 5. Independent factors associated to NAS-score > = 5 were Ms and BMI > 30. Leptin levels were higher in patients with advanced fibrosis (≥ F2) compared to patients with mild fibrosis (F0-F1) (75.5 + 50.2 ng/ml vs - 39.7 + 38.4 ng/ml respectively; p = 0.002).**

***Conclusion:* presence of Ms and obesity (BMI >30) are the principal independent factors associated to NASH (NAS score > = 5). Leptin levels and BMI are higher in patients with advanced fibrosis.**

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Key words: *Non alcoholic steatohepatitis. Metabolic syndrome. Adipokines. Body mass index.*

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FACTORES PREDICTIVOS DE ESTEATOHEPATITIS NO ALCOHÓLICA: PAPEL DEL SÍNDROME METABÓLICO

Resumen

La esteatohepatitis no alcohólica (EHNA) se ha propuesto como la manifestación hepática del síndrome metabólico (SM), con la resistencia a la insulina (IR) como mecanismo fisiopatológico común.

***Métodos:* se incluyeron 145 pacientes con biopsia hepática con enfermedad por hígado graso no alcohólica. NAS-score se utilizó para graduar la EHNA. Se realizaron las siguientes determinaciones; antropometría, presión arterial basal (BP), LDL colesterol, HDL colesterol, triglicéridos, leptina, resistencia a la insulina (HOMA-IR) y ecografía abdominal. El diagnóstico de síndrome metabólico se realizó en base a los criterios del ATP III.**

***Resultados:* la edad fue 43,6 + 11,2 años y la media de índice de masa corporal (IMC) 39 + 10.7 kg/m² (66 mujeres y 79 varones). Cuarenta pacientes (27,5%) presentaron una puntuación NAS> = 5. La circunferencia de la cintura (p = 0,007), la presión arterial sistólica y diastólica (p = 0,002 y p = 0,003, respectivamente, la resistencia a la insulina (HOMA-IR) (p <0,0001), IMC (p = 0,04), el SM (p = 0,04) y los datos ecográficos se asociaron significativamente con NAS-score > = 5. Los factores independientes asociados a NAS-score > = 5 fueron el SM y el IMC > 30. Los niveles de leptina fueron mayores en pacientes con fibrosis avanzada (≥ F2) en comparación con los pacientes con fibrosis leve (F0-F1) (75,5 + 50,2 ng / ml frente a 39,7 + 38,4 ng / ml, respectivamente; *p* = 0.002).**

***Conclusión:* la presencia de SM y obesidad (IMC> 30) son los principales factores independientes asociados a la EHNA (puntuación NAS> = 5). Los niveles de leptina y el IMC son mayores en los pacientes con fibrosis avanzada.**

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Palabras clave: *Esteatohepatitis no alcohólica. Síndrome metabólico. Adipokinas. Índice de masa corporal.*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is recognized worldwide as the most common cause of chronic liver disease. The spectrum of the disease ranges from simple steatosis, which has a more benign course to the histological severe steatohepatitis (NASH), a pathological entity associated with an increased risk for developing cirrhosis and hepatocellular carcinoma[1].

Insulin resistance (IR) is a condition in which the cells of the body become resistant to the effects of insulin[2]. IR can have important long-term consequences, including the development of type 2 diabetes mellitus (DM), cardiovascular disease, and certain malignancies that are associated with obesity and IR[3]. Fracanzani *et al.*[4] and Mofrad *et al.*[5] suggested that IR or diabetes could play a role in elevations of plasma alanine aminotransferase (ALT) levels in adults.

There is also an apparent association among IR, abdominal obesity, and a variety of abnormalities, such as type 2 DM, hypertension, an atherogenic lipid profile that includes hypertriglyceridemia and low serum high-density lipoprotein cholesterol (LDL/HDL-Chol) concentrations[2]. This constellation of disorders is called metabolic syndrome (Ms). Non-alcoholic fatty liver disease (NAFLD) has been proposed as the hepatic manifestation of the Ms, with IR as the common pathophysiological mechanism[6].

Additionally, the severity of insulin resistance correlates with liver histology in subjects with NAFLD[7,8,9].

However, NAFLD may also be encountered in non-obese, non-diabetic individuals[8].

The pathogenesis of NAFLD has not been fully elucidated. The most widely supported theory implicates IR as the key mechanism leading to hepatic steatosis, and perhaps also to steatohepatitis. Also, other multiple hits as hepatic iron, leptin, antioxidant deficiencies, and intestinal bacteria have all been suggested as potential oxidative stressors; treatment with probiotic agents has demonstrated to reduce transaminases levels in patients with NAFLD[10].

However, at the present time, given the large number of patients at risk (patients that are obese, have Ms, and/or type 2 DM), many clinicians still only consider a diagnostic liver biopsy in those with persistently elevated liver enzymes, when other liver condition have been excluded[11]. Yet, it is still unknown why some patients have high BMI and presence of Ms while others do not. Moreover, several genes have been identified to be associated with these phenotypes and they could explain different manifestations of the same disease[12-14].

The aim of this study is to evaluate the association of IR, Ms and BMI in relation to liver histology in patients with NAFLD.

Methods

Study population

We included a total of 145 individuals who were diagnosed of NAFLD using liver biopsy. These patients were remitted to our hospital due to elevations of transaminases levels. Exclusion criteria were daily alcohol intake > 20 g (men) or > 10 g (women), history of diabetes, fasting hyperglycemia, positive serologic finding for hepatitis B or C virus, history of malignancy, stroke, cardiovascular disease or hepatectomy, any chronic use of medications other than vitamins, abnormal findings on ultrasound as intrahepatic bile duct dilatation, intrahepatic stone, liver nodule or mass, and other ethiology factors of chronic liver disease. We determined anthropometric measurements and abdominal ultrasound scan (US). A fasting blood sample was used to measure plasma aminotranferases, serum insulin, glucose HOMA-IR, lipid profile and adipokines levels. All individuals had a US performed as a part of their study.

Measurement of variables

Anthropometric measurements: Their height, weight, and waist circumferences were measured to the nearest half-cm or half-kg. The waist circumference was measured at the mid-point between the lower border of the rib cage and the iliac crest.

Laboratory evaluation included measurement of the ALT, fasting blood glucose, fasting insulin, total, cholesterol, HDL-Chol, LDL-Chol and triglycerides. IR was determined by homeostasis model assessment index (HOMA–IR), calculated using the computer-based solution of the model provided by the Diabetes Trials Unit, Oxford Center for Diabetes, Endocrinology, and Metabolism[15]. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5m UI/L (normal range 0.5-30 mUI/L) and the HOMA was calculated using these values[16].

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL-Chol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml[17]. Adiponectin was measured by ELISA (R&D systems, Inc., Mineapolis, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml[18].

Definitions of Metabolic Syndrome

The diagnosis of Ms was based on the presence of three or more of the components listed by the revised Adult Treatment Panel (ATP III) of the National Cholesterol Education Program[15].

These components include: 1) elevated waist circumference (waist circumference >90 cm in males or > 85 cm in females); 2) elevated triglyceride (> 150 mg/dl); 3) reduced HDL-Chol (< 40 mg / dl for men and < 50 mg / dl for women); 4) elevated blood pressure (> 130/85 mm Hg or the use of medication for hypertension); 5) elevated fasting glucose (> 100 mg/dl or the use of medication for hyperglycemia).

Histology

Liver histology (H & E and Masson's trichrome stain) was centrally reviewed by the Pathology Committee of the NASH CRN blinded to clinical data. Biopsies were scored for the following features according to NASH CRN criteria published by Kleiner *et al*.[19]:

Steatosis (grade 0 (< 5% macrovesicular fat in hepatocytes), grade 1 (5–33%), grade 2 (34–66%), grade 3(>66%)), lobular inflammation (0–2),hepatocellular ballooning (0–2),and fibrosis (stage 0, stage 1a (mild perisinusoidal), stage 1b (moderate perisinusoidal), stage 1c (portal / periportal fibrosis only), stage 2 (zone 3 and periportal), stage 3 (bridging fibrosis), stage 4 (cirrhosis)). A NAFLD activity score (NAS-score) was tabulated by summing the scores for steatosis, lobular inflammation, and ballooning degeneration (1–8 score possible). For regression analyses, all fibrosis stage 1 (1a, 1b, and 1c) biopsies 2 were combined and treated as mild stage, and stage 3 and 4 biopsies were combined and treated as advanced stage, and a categorical NAS score was defined as: 0 = 1 –4 (reference), 1 = 5–8 NAS score of 0-2 occurred in cases largely considered not diagnostic of NASH; scores of 3-4 were divided among those considered not diagnostic borderline. Scores of 5-8 occurred in cases that were largely considered diagnostic of NASH[20,21].

Radiologic assessment

Findings of the ultrasonographic scan of upper abdomen were noted in all patients to assess the grade of fatty liver at presentation and to rule out other hepatic pathology. The severity of hepatic steatosis was graded in these patients based on previously published criteria[22].Ultrasonographically detected-NAFLD (US-NAFLD) was defined when the ultrasonography revealed fatty liver. Fatty liver were diagnosed with the ultrasound based on known standard criteria, including parenchymal brightness, liver-to-kidney contrast, deep beam attenuation, and bright vessel walls[22-23].

Statistic

Values were expressed as mean + SD or as a number (percentage).

The significance of the relationship between NASH and baseline characteristics or histology was determined from a Wilcoxon two-sample test for continuous variables, and either a χ^2 test for non-ordered categories, a Cochran-Armitage trend test for ordered categories. Fisher's exact test, or binary or multinomial logistic regression for categorical variables. Adjusted odds ratios or cumulative odds ratios of categorical histological feature with the presence of a component of MS were estimated from either a logistic (for binary outcomes), multinomial or ordinal logistic regression model. *P* values were determined from a likelihood ratio test. Regression models presented were adjusted for sex and age. A p-value <0.05 was considered significant. SPSS 15.0 (IL, USA) was used as statistical software.

Results

A total of 145 patients gave informed consents and were enrolled in the study. The mean age was 43.6 ±11.2 years old (range 18-67) an and the mean BMI 39±10.7 kg/m^2 (range 22-76) with 66 females and 79 males (Table I).

Table I
Clinical characteristics of 145 patients divided by NASH (NAS score >=5)

	NAS score <4 (no NASH)	*NAS score >=5*
Age (years)	44.2±10.6	43.4±12.4
Gender (n)(male/female)	55/24	50/16
WC (cm)	100±12.5	1.3.9±13
BP systolic (mmHg)	129.4±22.7	138.±22.5*
BP dyastolic (mmHg)	78.7±19.9	85.8±17.9*
HOMA-IR	3.4±2-6	4.6±2.9*
AST (IU/L)	35.4±22.9	39.6±25.6
ALT (IU(L)	52.9±41.6	67.9±41.9*
Total Chol (mg/dl)	181.8±52.1	186.4±39.3
Ldl-Chol (mg/dl)	110.6±41.7	111.9±34.7
Hdl-Chol (mg/dl)	43±13.8	45.5±26.4
BMI (Kg /m^2)	39.6±11.1	40.8±10.6
Leptin (ng/ml)	39.5±33	55.1±53.2*
Adiponectin (ng/ml)	14.9±19	13±20.8
Ms (n %)	44 (42%)	30 (75%)*

WC: waist circumference, TG: triglicerides, Total ch: total cholesterol, BP: blood pressure, AST: aspartate amino transpherase, ALT: alanine amino transpherase, BMI: body mass index, Ms: metabolic syndrome.

Serum albumin, iron parameters and prothrombin time was with the normal range in all patients.

The US examination of the abdomen revealed a fatty liver in 72 (50%) patients. None of the patients had features of portal hypertension.

Forty patients (27.5%) patients were classified as NASH (NAS score 5-8). The histopathologic features of patients are given in table II.

Univariate analysis results revealed that waist circumference (p=0.007), blood pressure systolic/diastolic ratio (p=0.001), HOMA-IR (p<0.0001), BMI (p=0.04), Ms (p=0.05) and US-NAFLD were significantly associated with NAS>=5 (p<0.005) (Table III). Figure 1 shows relation between US-NAFLD findings and NAS score.

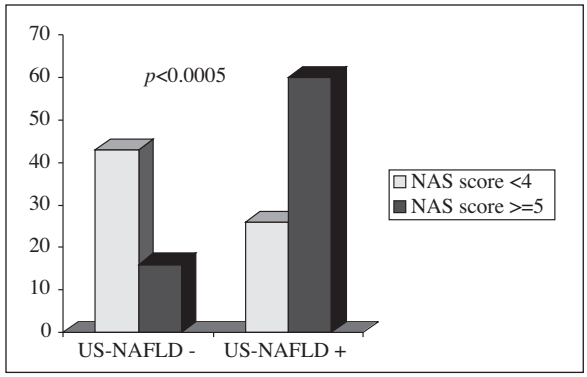

Fig. 1.–Relation between US-NAFLD findings and NAS score.

Table II
Histologic characteristics (n=145)

Histological parameter	*Frequency n(%)*			
	Grade 1	*Grade 2*	*Grade 3*	
Steatosis	35(24.1%)	36(24.8%)	74(51%)	
Balloning	128(88.2%)	17 (11.7%)	-	
Lobular inflammation	48(33.1%)	58 (40%)	39 (26.8%)	
Fibrosis	Stage 0 68(46.8%)	Stage 1 58(40%)	Stage 2 9(6.2%)	Stage 3 10(6.8%)

Table III
Binary Lineal regression between independent factors and "definitive NASH" (score 5-8)

	OR	*I.C. 95% para OR*		*Sig.*
		Inferior	*Superior*	
Age	1.013	.985	1.041	0.366
Gender	1.189	.645	2.192	0.579
WC (cm)	1.065	1.017	1.115	0.007
TG (mg/dl)	1.003	.998	1.008	0.250
BP systolic	3.316	1.638	6.713	0.001
BP dyastolic	3.214	1.739	5.958	0.001
HOMA-IR	1.401	1.168	1.681	0.000
AST (UI/L)	1.004	.991	1.017	0.543
ALT (UI/L)	1.001	.994	1.009	0.699
Total Ch (mg/dl)	1.001	.995	1.008	0.661
Ldl-Ch (mg/dl)	1.003	.994	1.011	0.530
Hdl-Ch (mg/dl)	.996	.978	1.015	0.675
BMI	1.032	1.001	1.064	0.042
Leptin (ng/ml)	1.008	0.998	1.018	0.048
Adiponectin (ng/ml)	.989	.971	1.007	0.233
Ms	2.815	1.367	5.800	0.005

WC: waist circumference, TG: triglicerides, Total ch: total cholesterol, BP: blood pressure, AST: aspartate amino transpherase, ALT: alanine amino transpherase, BMI: body mass index, Ms: metabolic syndrome.

Table IV
Multivariate analysis of association between NASH (NAS score >=5) and its independent predictors

	OR	*I.C. 95% para OR*		*p*
Ms	2.248	1.030	4.906	.042
BMI>30	2.436	1.077	5.506	.032

Multivariate analysis adjusted for gender and age including independent significant variables revealed that the only independent predictors of NAS >= 5 were Ms and BMI > 30 (Table IV).

Factors associated with fibrosis stage

An evaluation was carried out to identify factors associated with severe fibrosis (stages 2,3,4 vs stages 0 and 1 fibrosis). Univariate analysis revealed that HOMA-IR (3.6+2.6 vs 5.1+3.4; p=0.03), BMI (38.1+10.4 Kg/m^2 vs 45.6+10.1 Kg/m^2;p =0.004), and leptine levels (39.7+38.4 ng/ml vs 75.5+50.2 ng/ml; p=0.002) were significantly associated with degree of fibrosis. Multivariate analysis adjusted for gender and age showed that the independent predictors of advanced fibrosis were leptin levels and BMI (Table V).

Discussion

In this study we have identified the factors associated to more advanced degree of NAFLD were central obesity determined by WC, BP systolic and dyastolic, HOMA-IR, BMI, and Ms. However, multivariate analysis showed that independent predictors of definitive NASH were Ms and BMI>30.

Non-alcoholic fatty liver disease represents the hepatic manifestation of the Ms. NAFLD pathological alterations, which range from simple steatosis to steatohepatitis, may lead to fibrosis and end stage liver disease[24]. As its incidence parallels that of the Ms, NAFLD is currently becoming one of the first chronic liver diseases in Western countries and therefore has a major health impact[25].

In the same way, central obesity, which reflects visceral adiposity, it is often measured using the BMI and various cut off values are proposed[26,27,28]. Yet, BMI does not allow distinguishing central obesity, which is a metabolic disorder included in the MS, from peripheral obesity. In that sense, waist circumference appears to be more reliable and should be preferred[28].

Several studies have demonstrate a correlation between BMI, steatosis and NASH[29,30]. Elevated splachnic serum free fatty acid levels and reduced hepatic insulin clearance play a role in the pathogenesis of diabetes, hyperlipedemia and hypertension in patients with central obesity.

The prevalence of US-NAFLD in this study was 51.1% which was significantly higher in patients with NAs score 5-8 (78.9%) in comparison with US-NAFLD in patients with NAs scores <5 (21.1%). These results are in agreement with results of study published by Sinn DH *et al*.[31]

The effect of BMI is similar to the results of Rocha and Fassio, and we suggested that BMI measurement is helpful for evaluation of NAFLD[32,33]. As well as previous studies, BMI is predictor of NAFLD severity or it is significantly higher in the patients with fatty liver[34,35].

In our study we have observed a significant association between leptine levels and fibrosis stage. The relationship between leptin and hepatic fibrosis is controversial. Studies in vitro have clearly demonstrated a role in profibrogenic responses within the liver[17]. In accordance with our study, patients with NAFLD, and alcoholic liver disease have increased levels of circulating leptin[36]. In NASH, leptin receptors become resistant to its effect leading to hyperleptinemia which alters insulin signaling and promotes accumulation of intracellular fatty acids in hepatocytes thereby increasing hepatic steatosis and steatohepatitis[36]. Our study indicates that serum leptin levels are associated to advanced fibrosis in patients with NAFLD but it is not an independent predictor of NASH severity (evaluated by NAS score)[37]. Similar findings have been reported previously[38].

Leptin is strongly associated with Ms and its components, which are indicators of metabolic disorders such as dyslipidemia, DM2, and hypertension[39].

In conclusion presence of Ms and obesity (BMI >30) are the principal independent factors associated to definitive NASH (NAS score >=5). Leptin levels and BMI are higher in patients with avanced fibrosis. A US-NAFLD positive result is usefull to detect patients with NAFLD.

Tabla V
Multivariate analysis of association between fibrosis stage (mild: stage 0,1 vs. advanced: stage 2,3,4) and its independent predictors

	Regression coefficient	*St error*	*t value*	*Significance*	*95% confidence interval*
Leptine levels	0.4	0.02	1.05	0.009	1-1.2
BMI	0.4	0.2	1.6	0.01	1.1-2.8

Of course, for confirmation of this association, more comprehensive studies are needed.

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