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ORIGINAL ARTICLE

Non Alcoholic Fatty Liver Disease: Correlation With Clinical Hormonal and Biochemical Parameters

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ABSTRACT

BACKGROUND: Non alcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis (NASH) and cirrhosis. The aim of this study was to describe the variance of clinical and laboratory parameters that could play a role in the development of NASH in a group of patients with NAFLD.

PATIENTS AND METHODS: Clinical, biochemical and histological features of 36 patients with NAFLD were analyzed. Insulin and leptin serum levels were measured and Body Mass Index (BMI) and insulin resistance (HOMA index) were calculated. The same parameters were analyzed in 10 healthy controls.

RESULTS: Signs of chronic liver disease and histological findings of advanced fibrosis were detected in 2 patients (5,5%), while hepatomegaly was a frequent finding (21 patients, 58,3%). NASH was confirmed in 21 patients (58,3%).Statistically significant difference was found between patients and controls in BMI (32 vs 25, p=0,002), insulin levels (33,3 vs 9,5, p=0,015) and HOMA index (9,4 vs 2,3 p=0,006). In comparing NASH patients with fatty liver (FL) patients, we observed similar levels (31,1 vs 32) of BMI, while insulin levels were higher in NASH patients (42 vs 22 p=0,044), as well as HOMA (11 vs 7,5) and leptin levels (22 vs 16,6) although this differences did not reach a statistical significance.

CONCLUSIONS: This study confirms the important role of obesity and insulin resistance in a group of greek NAFLD patients. A positive correlation is also found between severity of liver damage and degree of insulin resistance.

List of Abbreviations:
NAFLD: Non alcoholic fatty liver disease
NASH: Steatohepatitis
BMI: Body Mass Index
FL: Fatty liver
HOMA: Homeostatic Model Assessment

KEY WORDS: *non alcoholic fatty liver disease, steatohepatitis, leptin serum levels, insulin resistance*

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INTRODUCTION

NAFLD is an increasingly recognized condition that refers to a wide spectrum of liver damage, ranging from simple fatty liver to steatohepatitis, which can progress to advanced fibrosis and cirrhosis. NAFLD should be differentiated from steatosis resulting from secondary causes such as genetic disorders (lipodystrophy, dysbetalipoproteinemia, etc), nutritional disorders (starvation, total parenteral nutrition, protein caloric malnutrition etc), drugs (estrogens, glucocorticoids, amiodarone, valproic acid

etc) environmental hepatotoxins, HIV infection, and inflammatory bowel disease. (1-6)

NAFLD is mainly associated with obesity, diabetes mellitus (type 2), hyperlipidemia and insulin resistance, which are the main features of the metabolic syndrome. The pathogenesis of NAFLD is poorly understood, as well as why simple steatosis develops in some patients, whereas steatohepatitis and progressive disease in others. The "multi-hit" theory suggests that in the first "hit", insulin resistance leads to the accumulation of fat within hepatocytes by two main mechanisms: lipolysis and hyperinsulinemia. The second "hit" involves oxidative stress resulting from mitochondrial release of reactive oxygen species, lipid peroxidation, cytokine induction, and the induction of Fas ligand. In addition, recent data suggest a potential role for leptin - a hormone that regulates body weight through a hypothalamic mechanism – in the pathogenesis of NAFLD and NASH by inducing dephosphorylation of insulin-receptor substrate-1. (3,6-9)

It is also worth noting that, although, NASH was originally considered to occur almost exclusively in obese patients, it is now known to affect even those with normal body weight.(4,6) Furthermore only 10-30% of NAFLD patients have biochemical and histological findings of NASH an important distinction since NASH can result in progressive liver disease while steatosis alone may not. (6,10)

STUDY AIM

The aim of this study was to measure the prevalence and variance of clinical and laboratory parameters in NAFLD patients and to detect differences of their levels between NASH patients and patients with fatty liver alone, in order to verify the association of certain factors – such as the degree of insulin resistance, BMI, serum leptin levels – with the development of the potentially progressive NASH.

PATIENTS AND METHODS

PATIENTS

The study was performed in 36 patients, referred to the liver unit of our department in the period 2004 to 2006, with the following characteristics:

(1) Liver steatosis confirmed by ultrasound. Brightness and posterior attenuation were considered indices of the extent of fatty infiltration.
(2) Negative or negligible alcohol consumption and no hepatotoxic medication.
(3) Negative hepatitis B and C viral markers, absence of autoantibodies indicative of autoimmune liver disease, and normal ferritin and a1-antithrypsin levels.

The control group included 10 healthy individuals (5 men and 5 women selected to have the same mean age with the patients), with no liver disease history and a normal liver ultrasound. They have been submitted in the same, as the patients, clinical and laboratory tests.

All the subjects gave informed consent for participation in the study.

METHODS

A complete family and personal medical history of all patients was recorded and a complete physical examination was performed. Body weight and height were measured and BMI was calculated in all participants. Laboratory studies included glucose, creatinine, cholesterol, triglyceride, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), albumin and total protein levels; hepatitis B surface antigen and antibody to hepatitis C virus; autoimmune serology; studies of iron metabolism, ceruloplasmin and a1-antitrypsin levels. All the patients underwent a glucose tolerance blood test, and insulin and leptin were measured by immunoenzymometric assays (Cayman Ch.). Insulin resistance was calculated by means of the homeostasis model assessment HOMA as follows: HOMA-Index = [fasting insulin (mU/L) x fasting glucose (mmol/L)]/22,5.

The patients underwent a liver biopsy, after an informed consent was obtained and they were distincted in patients with steatohepatitis (NASH) and patients with simple liver steatosis (FL), according to Brunt's histological criteria. (11)

Statistical analysis. All data were analyzed using SPSS, and were compared across study groups by use of analysis of variance ANOVA.

RESULTS

1. CHARACTERISTICS OF PATIENTS AND SEVERITY OF LIVER DISEASE

Demographic and laboratory characteristics of the patients are shown in table I. Nine of the patients (25%) were diabetic while glucose tolerance test was abnormal in 17 patients (47,2%).

TABLE I. Characteristics of 36 NAFLD patients and healthy controls.

	Gender (F/M)	Mean Age (yr)
NASH* (n=21)	10/11	48,6 (28-73)
FL* (n=15)	8/7	48,5 (36-69)
NAFLD* (n=36)	18/18	48,6 (28-73)
Controls (n=10)	4/6	46,6 (35-58)

* FL: Fatty Liver, NAFLD: Non-Alcoholic Fatty Liver Disease, NASH: Non-Alcoholic Steatohepatitis.

Biochemical and histological findings of NASH were detected in 21 patients (58,3%), the remaining 15 were diagnosed as having simple fatty liver (FL). Chronic liver disease stigmata with histological findings of advanced fibrosis have been detected only in 2 (5,5%), both patients in the NASH group.

2. STATISTICAL DIFFERENCES BETWEEN PATIENTS AND CONTROLS

Comparison of the group of patients with the group of healthy controls (Table-II), demonstrated a statistically significant difference in:

- BMI (32 kg/cm^2 in patients, 25 kg/cm^2 in controls, p=0,002)
- Insulin serum levels (33,3 μIU/ml vs 9,5 μIU/ml respectively, p=0,015)
- Insulin resistance HOMA (9,4mg/l vs 2,3mg/l, p=0,006)
- ALT (mean values 45,2 U/L vs 17,7 U/L, p=0,002)
- Serum leptin levels did not differ significantly between the two groups.

3. STATISTICAL DIFFERENCES BETWEEN NASH PATIENTS AND PATIENTS WITH SIMPLE STEATOSIS (FL)

As shown in Table III, BMI was comparable between the group of NASH patients and that with fatty liver (31,1 kg/cm^2 vs 32 kg/cm^2 respectively). Insulin serum levels were significantly higher in NASH patients (42 μIU/ml vs 22 μIU/ml respectively, p=0,04). Serum leptin levels were higher in NASH patients (22 ng/ml vs 16,6 ng/ml), as well as insulin resistance HOMA index (11 vs 7,5) but the differences were not statistically significant. Serum lipid levels did not differ between the two groups. Mean values of ALT were higher in NASH patients (59,6 vs 25 , p<0,0001).

DISCUSSION

The present report on a group of greek patients, confirms that NAFLD is strongly associated with obesity, impaired glucose metabolism- hyperinsulinemia and insulin resistance. These findings agree with those of other studies, and confirm the presence of these associations in the greek population.(12,13) Since Greece holds one of the highest ranks in obesity (women 18,2% - men 26%) among the European countries (14), the impact of those morbid conditions in terms of public health may be of great importance.

The comparison of NASH patients with those with simple steatosis, indicates that the most important risk factor of progressive liver disease is high insulin levels, indicating insulin resistance, a finding in agreement with the results of other studies. (8,15,16) We assume that, although HOMA index was found higher in NASH patients (10,8 vs 7,5), this difference did not reach statistically significant levels because of the relatively small number of patients in each group. BMI and serum lipid levels were not found to correlate with the progression of liver damage to steatohepatitis in these patients. A possible explanation is that although obesity and hyperlipidemia are important risk factors of liver steatosis, they do not lead to progressive liver damage if insulin resistance does not coexist in the same patient.

In this study, although levels of serum leptin were found higher in NASH patients (22 ng/ml vs 16,6 ng/ml) they did not confirmed a statistically significant difference, as in other studies possibly due to the relatively small sample of patients. (17-20) However results of recent reports, regarding the role of leptin in NAFLD, are controversial (21-23) though in one study liver steatosis regressed in patients with severe lipodystrophy. (24)

In conclusion, the results of the present study in a group of greek patients, indicate that beyond the well known risk factors for NAFLD as obesity and hyperlipidemia – factors important for the greek population – insulin resistance holds the major role in the progression of the disease to steatohepatitis. The probable role of elevated serum leptin levels in promoting liver steatosis and NASH needs further investigation.

TABLE II. BMI, HOMA, Serum Insulin and leptin levels, in patients and controls.

	Patients	Controls	p
BMI	31,44	25,3	0,002
Insulin	33.34	9,5	0,015
HOMA	9,35	2,3	0,006
Leptin	19,7	20	0,95
ALT	45,2	17,7	0,002

TABLE III. Clinical and Biochemical markers in NASH patients and patients with simple steatosis (FL).

	NASH (n=21)	FL (n=15)	p
BMI	31,1	32	0,62
Insulin	41,9	21,9	0,044
HOMA	10,8	7,5	0,2
Leptin	21,9	16,6	0,32
Triglycerides	153	210	0,2
Cholesterol	235	231	0,8
HDL Cholesterol	44,3	44,8	0,9
ALT	59,6	25	0,0001

REFERENCES

1. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Steatohepatitis. *Mayo Clin Proc* 1980;55: 434-438.
2. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990;12:1106-1110.
3. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; 346:1221-31.
4. Bacon BR, Farahvash MJ, Janey CG, Neuschwander-Tetri BA. Nonalcoholic Steatohepatitis: An Expanded Clinical Entity. *Gastroenterology* 1994;107:1103-1109.
5. Powell EE, Graham E, Cooksley W, Hanson R et al. The natural history oh non-alcoholic Steatohepatitis: A follow-up study of forty two patients for up to 21 years. *Hepatology* 1990; 11:74-80.
6. Sanyal AJ. AGA technical review on non-alcoholic fatty liver disease. *Gastroenterology* 2002; 123:1705-1725.
7. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med* 2000; 343:1467-76.
8. Chitturi S, Farrell GC. Etiopathogenesis of non-alcoholic steatohepatitis. *Semin Liver Dis* 2001;21:27-41.
9. Le D, Marks D, Lyle E, Corless CL et al. Serum leptin levels, hepatic leptin receptor transcription, and clinical predictors of non-alcoholic steatohepatitis in obese bariatric surgery patients *Surg Endosc* 2007.
10. Younossi Z, Diehl AM, Ong JP. Nonalcoholic fatty liver disease: An agenda for clinical research. *Hepatology* 2002;35:746-752.
11. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetr BA et al. Non alcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;94:2467-2474.
12. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB et al. Nonalcoholic Steatohepatitis: Association of Insulin resistance and Mitochondrial abnormalities. *Gastroenterology* 2001;120: 1183-1192.
13. Kawasaki T, Hashimoto N, Kikuchi T, Takahashi H, et al. The relationship between fatty liver and hyperinsulinemia in obese japanese children. *J Pediatr Gastroenterol Nutr* 1997;24:317-321.
14. Greek Obesity Society, EASO European Symposium, Praga 2004.
15. Marchesini G, Bugianesi E, Forlani G, Cerrelli F et al. Nonalcoholic Fatty liver, Steatohepatitis and the Metabolic syndrome. *Hepatology* 2003;37: 917-923.
16. Bugianesi E, Manzini P, D'antico S, Vanni E et al. Relative contribution of iron burden, HFE mutations and insulin resistance to fibrosis in non-alcoholic fatty liver. *Hepatology* 2004;39: 179-87.
17. Tobe K, Ogura T, Tsukamoto C, Imai A et al. Relationship between serum leptin and fatty liver in Japanese male adolescent university students. *Am J Gastroenterol* 1999; 94: 3328-3334.
18. Uygun A, Kadayifci A, Yesilova Z, Erdil A et al. Serum leptin levels in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2000;95(12): 3584-3588.
19. Chitturi S, Farrell G, Frost L, Kriketos A et al. Serum Leptin in NASH correlates with hepatic Steatosis but not Fibrosis:A manifestation of Lipotoxicity. *Hepatology* 2002;36: 403-409.
20. Nobili V, Manco M, Ciampalini P, Diciommo V et al. Leptin, free leptin index, insulin resistance and liver fibrosis in children with non-alcoholic fatty liver disease. *Eur J Endocrinol* 2006; 155(5):735-43.
21. Serin E, Ozer B, Gumurdulu Y, Kayaselcuk F et al. Serum leptin level can be a negative marker of hepatocyte damage in nonalcoholic fatty liver. *Am J Gastroenterol* 2003; 38: 471-6.
22. Chalasani N, Crabb DW, Cummings OW, Kwo PY et al. Does leptin plays role in the pathogenesis of human nonalcoholic steatohepatitis? *Am J Gastroenterol* 2003; 98: 2771-6.
23. Lydatakis H, Hager IP, Kostadelou E, Mpousmpoulas S et al. Non-invasive markers to predict the liver fibrosis in non-alcoholic fatty liver disease. *Liver Int* 2006;26(7):864-71.
24. Petersen KF, Oral AE, Dufour S, Befroy D et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 2002;109:1345-50.