Coll. Antropol. **34** (2010) Suppl. 1: 33–37
Original scientific paper

Predictors of Nonalcoholic Steatohepatitis in Patients with Elevated Alanine Aminotransferase Activity

Željko Puljiz[1], Davor Štimac[2], Dražen Kovač[3], Mario Puljiz[4], Andro Bratanić[1], Vedran Kovačić[1], Duško Kardum[5], Damir Bonacin[1] and Izet Hozo[1]

[1] Department of Gastroenterology, University Hospital Center Split, Split, Croatia
[2] Department of Gastroenterology, University Hospital Center Rijeka, Rijeka, Croatia
[3] Clinical Institute of Pathology, University Hospital Center Rijeka, Rijeka, Croatia
[4] University Hospital for Tumors, Zagreb, Croatia
[5] Institute of Gastroenterology, University Hospital Dubrava, Zagreb,Croatia

A B S T R A C T

Incidence of obesity and hepatic steatosis is increasing worldwide. Almost one quarter of western countries population suffer from non alcoholic fatty liver disease (NAFLD). The aim of this study was to investigate the frequency and predictors of nonalcoholic steatohepatitis (NASH) in patients with unexplained alanine aminotransferase activity elevation (ALT), and therefore avoid unnecessary biopsies in cases of simple steatosis. Earlier studies provided different results and have not answered the question how to distinguish NASH from simple steatosis. Ultrasound (US), computed tomography (CT) and magnetic resonance (MRI) can detect steatosis with great sensitivity level, but not NASH. This study included 50 patients (18 women and 32 men) with mean age 43±9 years, and with defined selected biochemical, anthropometric and hormone biomarkers. The average BMI was 27.1±3.81 (kg/m^2), insulin resistance HOMA IR 3.89± 3.81. All patients underwent liver biopsy and NASH was staged by NASH activity score (NAS) from 1 to 8. Results are compared to pathohistological finding as relevant method. The results show that 90% of patients (n=45) had NAFLD (minimal stage at least), and 15 (30%) had nonalcoholic steatohepatitis (NASH). High triglyceride, low HDL and high ferritin serum levels correspond with NASH. As in earlier studies, insulin resistance as basic mechanism of NAFLD and NASH was confirmed.

***Key words**: fatty liver, steatohepatitis, metabolic syndrome, insulin resistance*

Introduction

Liver cirrhosis is frequent cause of death in western countries[1]. Although chronic B and C hepatitis, as well as alcoholism, are important causes of chronic liver disease, in many patients etiology is still unclear[1]. Today, it is known that non-alcoholic fatty liver disease (NAFLD) is cause of substantial number of liver cirrhosis of unknown origin (cryptogenic cirrhosis). NAFLD frequency in general population is not precisely known, but is considered one of most frequent liver diseases, and strikes up to 24% of population[1–3]. NAFLD is defined as liver component of metabolic syndrome which includes hyperlipidemia, hypertension, obesity and glucose intolerance[4,5]. Histological characteristics of NAFLD are similar to liver disease caused by alcohol; from steatosis (fatty liver) and steatohepatitis (steatosis with parenchymatous inflammation with or without focal necrosis) to different stages of fibrosis, including cirrhosis[6]. NAFLD activity score (NAS) is in wide use[7]. Steatosis is mostly macrovesicular, evenly distributed in lobuli. NASH is, in fact, part of spectrum of NAFLD, more precisely, its advanced form. It comprises increased alanine transaminase activity (ALT) and histological appearance of alcohol hepatitis, but without alcohol usage in anamnesis[8]. A »Two hit« theory was accepted as possible pathogenetic

Received for publication May 5, 2009

TABLE 1
OUTPUT MEASURES FOR ALL SUBJECTS

	$\overline{X}$±SD	Minimum	Maximum
ESR (mm/h)	11.00±12.20	1.00	65.00
CRP (mg/L)	2.31±2.26	0.00	11.50
Leukocyte x10/L	6.00±1.66	3.70	10.50
Thrombocyte x10 /L	218.46±56.62	2.00	350.00
Erythrocyte x10/L	4.66±0.50	3.80	5.90
Hemoglobin (g/L)	139.50±13.70	117.00	179.00
P.V. (%)	58.04±44.19	1.00	116.00
Fe (μmol/L)	19.59±6.57	4.80	41.70
UIBC (μmol/L)	38.80±9.45	25.00	72.00
TIBC (μmol/L)	57.80±7.79	36.00	79.50
Ferritin (μg/L)	204.48±146.18	16.00	599.00
Bilirubin (umol/L)	16.49±8.43	6.40	48.30
Total cholesterol (mmol/L)	6.30±1.68	3.20	11.80
Triglyceride (mmol/L)	1.89±1.37	0.60	9.00
HDL (mmol/L)	1.25±0.62	0.60	5.00
IgG (g/L)	12.60±4.31	6.30	33.20
Fasting glucose (mmol/L)	5.91±1.67	3.90	11.80
HbA1c (%)	5.39±1.01	3.70	8.20
Creatinine (μmol/L)	84.88±17.30	56.00	154.00
AST (U/L)	70.38±83.16	25.00	586.00
ALT (U/L)	118.40±100.82	41.00	674.00
GGT (U/L)	135.80±84.80	10.00	450.00
AP (U/I)	83.39±28.90	39.00	185.00
LDH (U/L)	222.06±75.05	123.00	423.00
CK (U/L)	86.88±44.39	32.00	248.00
Total protein (g/L)	73.56±5.13	58.00	84.00
Albumin (g/L)	47.46±6.41	26.00	62.00
BMI (kg/m^2)	27.18±3.10	23.00	34.00
AST/ALT	0.59±0.21	0.28	1.39
Insulin (μU/mL)	14.02±11.78	2.30	72.10
HOMA	3.83±3.41	0.60	19.50

$\overline{X}$±SD: mean±standard deviation; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; HDL, high-density lipoprotein; CK, creatine kinase; BMI, body mass index; HOMA, homeostasis model assessment for insulin resistance=fasting glucose (mmol/L) * fasting insulin (μmol/mL)/22.5

mechanism so far[9]. Increased trigliceride and free fatty acids accumulation in liver causes steatosis (first hit), and steatosis progression leads to steatohepatitis (NASH), along with other factors such as oxidative stress, mitochondrial damage, fatty acids lipotoxicity (second hit)[9]. Insulin resistance and hyperinuslinemia are primary pathogenic factors in steatosis itself, besides eventual glucose intolerance[10–12].

Patients with NASH are most often without any symptoms, but are by chance diagnosed with elevated ALT activity[8]. Aspartate aminotransferase (AST) and gamma glutamil-transpeptidase (GGT) activities can be elevated as well[8]. Of all enzymes, ALT is connected to fat accumulation the most, and therefore it is used as fat liver disease marker[13]. Presence of liver steatosis in patients with metabolic syndrome and unexplained ALT el-

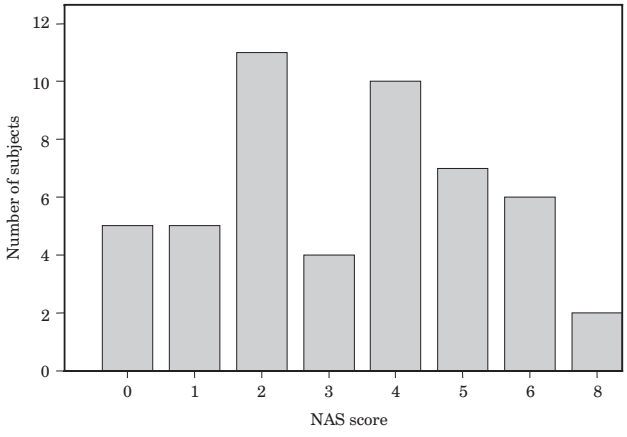

Fig. 1. Distribution of participants according to NAS score.

TABLE 2
DIFFERENCES BETWEEN SUBJECTS WITH NAS SCORE ≥5 (N=15) AND NAS SCORE <5 (N=35), STUDENT'S T-TEST FOR INDEPENDENT SAMPLES, ONE TAILED

	NAS ≥5 $\overline{X}$±SD	NAS<5 $\overline{X}$±SD	p
ESR (mm/h)	7.67±7.18	12.43±13.65	0.105
CRP (mg/L)	3.07±2.82	1.99±1.93	0.061
Leukocyte x10/L	6.52±1.89	5.78±1.52	0.076
Thrombocyte x10 /L	221.40±76.60	217.20±46.89	0.407
Hemoglobin (g/L)	143.80±17.32	137.66±11.63	0.074
P.V. (%)	58.15±43.96	58.00±44.93	0.496
Fe (μmol/L)	19.00±5.21	19.84±7.13	0.342
UIBC (μmol/L)	41.82±10.76	37.51±8.68	0.071
Ferritine (μg/L)	309.44±146.03	159.50±122.78	<0.001*
Bilirubin (umol/L)	18.04±9.73	15.83±7.87	0.201
Total cholesterol (mmol/L)	5.93±0.97	6.46±1.89	0.153
Triglyceride (mmol/L)	2.46±2.06	1.64±0.87	<0.026*
HDL (mmol/L)	0.98±0.19	1.36±0.70	<0.023*
IgG (g/L)	12.19±3.50	12.78±4.65	0.332
Fasting glucose (mmol/L)	6.22±1.76	5.78±1.63	0.200
HbA1c (%)	5.27±0.85	5.44±1.07	0.298
Creatinine (μmol/L)	87.33±12.77	83.83±18.99	0.259
AST (U/L)	60.87±4.842	74.46±95.68	0.301
ALT (U/L)	118.40±66.41	118.40±113.29	0.500
GGT (U/L)	130.00±112.43	138.29±71.68	0.378
AP (U/I)	81.73±24.23	84.12±31.04	0.397
LDH (U/L)	227.67±82.01	219.66±73.00	0.367
CK (U/L)	95.87±51.43	83.03±41.23	0.177
Total protein (g/L)	73.00±6.71	73.79±4.37	0.311
Albumin (g/L)	47.73±6.12	47.35±6.62	0.424
BMI (kg/m^2)	31.07±2.43	25.51±1.38	<0.001*
AST/ALT	0.52±0.15	0.62±0.22	0.053
Insulin (μU/mL)	23.66±16.54	9.89±5.37	<0.001*
HOMA	6.49±4.37	2.69±2.12	<0.001*

$\overline{X}$±SD: mean ± standard deviation, *: p<0.05; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; HDL, high-density lipoprotein; CK, creatine kinase; BMI, body mass index; HOMA, homeostasis model assessment for insulin resistance=fasting glucose (mmol/L)
* fasting insulin (μmol/mL)/22.5

evation is diagnosed reliably by US, CT, MRI, and confirmed by liver biopsy[14]. Consistency of US and liver biopsy diagnosed liver steatosis results are very weak (47% sensitivity; 63% specificity; 11% positive predictive value)[15]. On the other hand, negative predictive value of US results is significant (92%), and with steatosis in over half of hepatocytes, it rises to 96%[15].

Patients and Methods

50 patients were included in this prospective study; 32 (64%) males and 18 (36%) females, mean age 43±9 years with elevated amino-transferase unclear origin. Criteria for including was ALT>45 UI/L measured twice in last six months. We excluded from our study all patients with alcohol consumption of more than 20 g *per* week, with hepatic disease of known origin (cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, actual or past viral hepatitis B or C, autoimmune liver disease, Wilson's disease, hereditary hemochromatosis, α-1 antitrypsin deficiency) thyroid gland disease, hepatotoxic drugs therapy. Written informed consent was obligate for all participants. This study has obtained permit by Hospital Ethics Committee in accordance with Helsinki Declaration and lasted from January 2008 to December 2008 at the Department of Gastroenterology and Hepatology of the University Hospital Centre Split, Split and University Hospital Dubrava Zagreb, Croatia. Laboratory tests consisted of erythrocyte sedimentation rate (ES), CBC, serum liver function tests: aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), γ-glutamil transpeptidase (GGT), lactate dehydogenase

(LDH), bilirubin, creatine-phosphokinase (CPK), alkaline phosphatase (AP), prothrombin time (PT), iron studies, fasting triglycerides and cholesterol profile, HDL, LDL, ferritin, fasting glucose and insulin, glycolysed hemoglobin A1c (HbA1c), protein, albumin, AST/ALT, C reactive protein (CRP), creatinine, immunoglobulin G and A (IgA and IgG). Insulin resistance with HOMA IR score was calculated (fasting glucose (mmol/L)×fasting insulin (μU/mL)/22.5), body mass index (BMI). Arterial hypertension was assessed when RR was 140/60 mmHg or more. Ultrasound (US) was performed with Toshiba SSA-325A. All patients underwent ultrasound guided liver biopsy. Quality of the biopsy material was assessed macroscopically. Two pathologists separately made patohystological score of the nonalcoholic fatty liver disease with NAFLD Activity Score (NAS)[16]. NAS score includes different stages of steatosis, lobular inflammation and ballooning. Steatosis stage 0 (<5% cells), stage 1 (5– 33%), stage 2 (33–66%), stage 3 (>66%). Lobular inflammation: stage 0 (none), stage 1 (<2 foci/200x field), stage 2 (2–4 foci/200x field), stage 3 (>4 foci/200x field). Ballooning: stage 0 (none), stage 1 (few cells) and stage 2 (many cells/prominent ballooning). Sum of each components NAS ≥5 mean NASH, NAS <3 no evidence of NASH and NAS score 3 or 4 borderline/possible. The data were demonstrated as $\overline{X}$±SD. The difference between two groups was tested with Students t-test for independent samples. The correlation between variables was tested with Spearman's correlation test. The level of $p<0.05$ was considered statistically significant. The SPSS 14.0 program was used for statistical analysis.

Results

All liver biopsies were adequate for pathohistological analysis. Out of 50 included patients (age 43±9), BMI was 27.18±3.1, 22 (36%) patients were with arterial hypertension. HOMA IR score is 3.83±3.41, ALT 118.4±100.82. All biochemical and clinical parameters are demonstrated in Table 1. Figure 1 shows the number of participants

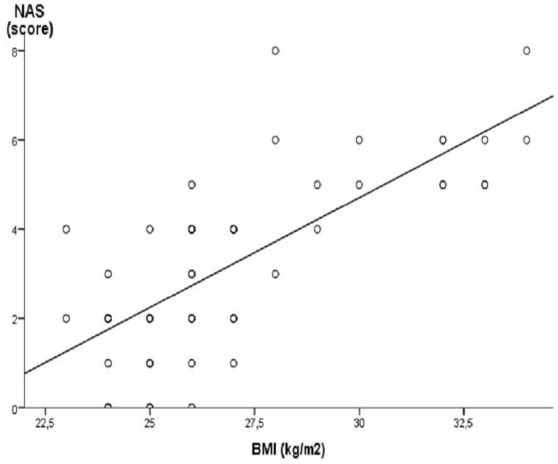

Fig. 2. Correlation between BMI and NASH score (r=0.733, p<0.001).

TABLE 3
STATISTICALLY SIGNIFICANT CORRELATIONS BETWEEN NAS SCORE AND BIOMARKERS AND CLINICAL PARAMETERS (SPEARMAN'S COEFFICIENT)

	Spearman's coefficient	p
Ferritin	0.42	0.001
HDL	–0.37	0.004
Plasma glucose	0.28	0.026
Creatinine	0.26	0.037
BMI	0.73	<0.001
Insulin	0.55	<0.001
HOMA	0.58	<0.001

HDL – high-density lipoprotein; BMI – body mass indeks; HOMA – insulin resistance=fasting glucose (mmol/L) * fasting insulin (μmol/mL)/22.5

depending on NAS score. Fifteen (30%) subjects had NASH. The subjects were divided in two groups depending on NAS score: NAS score≥5 and NAS score <5. Differences between two groups are demonstrated on Table 2. There were differences between NASH and non-NASH group in ferritin concentration (295.83±159.13 *vs*. 187.08±138.85; p=0.026), in BMI (31.38±2.45 *vs*. 26.38±2.53; p<0.001), in insulin plasma level (26.36± 19.59 *vs*.11.67±8.06; p<0.001), in triglyceride (2.46±2.06 *vs*.1.64±0.87; p<0.026), in HDL (high density lipoprotein) (0.98±0.19 *vs*. 1.36±0.70; p<0.023) and in HOMA (7.42±5.11 *vs*. 3.15±2.54; p<0.001). The NAS score was correlated with biomarkers and clinical parameters, and statistically significant correlations are demonstrated in Table 3. The strong positive significant correlation between NAS score and BMI is demonstrated in Figure 2 as a plot of linear regression.

Discussion

Obesity is a growing problem in the world with prevalence more than 20% of the USA population[16]. NASH, as advanced form of NAFLD is reversible. Today we have a liver biopsy as only reliable tool in diagnosis[4]. Special interest of many studies is to find biomarkers of NASH and distinguish it from steatosis. Prevalence of NASH in patients with chronic elevated amino alanine-transferase in our study is 30%, and is similar with others. Strong connection of some parameters as triglycerides, BMI, insulin levels is confirmation of crucial role of insulin resistance in pathogenesis of NASH. These results support the evidence that insulin resistance is an important element of NASH[17,18]. Obese patients with higher level of triglycerides commonly have hepatocyte necroinflamation. Insulin resistance is a sign of pre-diabetes and is accompanied by higher plasma insulin level. As a result of this data HOMA levels are higher in NAS group than in non-NASH. In our study 38% of patients have abnormal HOMA IR (>2.6). Lesser HDL as protection factor in NASH group shows that lobular necroinflammation is

relatively advanced liver disease which requires serious therapy in time, before developing heart disease. Early diagnosis is essential for preventing of cryptogenic cirrhosis and hepatocellular carcinoma. The finding that patients with cryptogenic cirrhosis are at risk of NASH now suggests that the end-stage disease may be greater problem than previously recognized. End stage of NAFLD liver disease is very common reason for liver transplantation. ALT is not a good predictor of NASH. Only patohistological diagnose can evaluate liver disease stage and prognosis, and biopsy remains gold standard. Different biomarkers in our study and elements of metabolic syndrome together are inexpensive and help us to detect NASH and make biopsy. Serum ferritin level has been reported significantly higher in NASH, which may reflect increased hepatic iron overload and enhanced oxidative stress[19]. We have shown that high BMI, hypertriglyceridemia (with decreased HDL), high level of plasma insulin and HOMA-IR can predict NASH. Biopsy in non-NASH group of patients may be postponed or avoided.

Conclusion

High levels of plasma insulin, triglycerides, ferritin with higher HOMA-IR and low HDL are good predictors of NASH.

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Ž. Puljiz

Department of Gastroenterology, University Hospital Center Split, 21000 Split, Croatia
e-mail: zpuljiz@sve.mo.ba

PREDIKTORI NEALKOHOLIČNOG STEATOHEPATITISA MEĐU PACIJENTIMA S POVEĆANOM AKTIVNOŠĆU ALANIN AMINOTARNSFERAZE

SAŽETAK

Debljina i nealkoholna masna bolest jetre su rastući svjetski problem. Gotovo četvrtina polulacije zapadnih zemalja pati od masne bolesti jetre. Cilj ove studije bio je odrediti učestalost i prediktore nealkoholnog steatohepatitisa u bolesnika s nejasnim porastom aktivnosti alanin-aminotransferaze. Na taj način bi mogli izbjeći biopsiju jetre u slučaju obične steatoze. Ranije studije su pokazale da ultrazvuk, kompjutorizirana tomografija i magnetska rezonanca pozdano detektiraju steatozu, ali ne i nekroinflamaciju. Naša studija je uključila 50 bolesnika, 18 žena i 32 muškarca. Svima su određeni različiti biokemijski antropometrijska i hormoski parametri te biopsija jetre. Svi rezultati su uspoređeni sa patohistološkim kao relevantnim. NASH je imalo 30% bolesnika. Dokazano je da su povišene vrijednosti triglicerida, feritina, inzulina i inzulinska rezistencij, uz visoki BMI pouzdani prediktori NASH-a. Rezultati studije nam omogućavaju da kombinacijom ovih parametara i ultrazvuka, izbjegnemo nepotrebne biopsije jetre.