Clinical, biochemical and histological correlations in a group of non-drinker subjects with non-alcoholic fatty liver disease

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Abstract

The correlation between biochemistry, imaging-studies and histology is a matter of controversy in non-alcoholic fatty liver disease (NAFLD) and the major pathophysiology of non-alcoholic steatohepatitis (NASH) is still unknown. We aimed to perform a comparative analysis between clinical, biochemical and histological variables of NAFLD. One-hundred and five NAFLD patients (F/M : 51/54), were studied, all with no-alcohol intake. The groups were followed-up for six months.

Necroinflammation and fibrosis were more severe in patients with diabetes (p = 0.002, and p = 0.0001, respectively). In comparing NAFL to NASH, plasma nitric-oxide and malondialdehyde levels were significantly higher (p = 0.05, for-both), and vitamin-E and-C levels were significantly lower in NASH (p = 0.002, and 0.001, respectively). The serum ferritin levels were higher in NASH patients (p = 0.016). While the ultrasonographic grade was significantly higher, the liver-spleen density gradient was significantly lower in NASH group (p = 0.017, and 0.005, respectively). Within a six month period, serum ALT levels dropped into the normal range in 23/76 (30.3%) patients and serum ALT in the 6th month correlated significantly with the severity of steatosis, inflammation and fibrosis in initial biopsy(p = 0.023, 0.035, 0.011, respectively).

In conclusion, the probability of severe liver disease is higher in patients with elevated-ALT in NAFLD. Serum ferritin levels have some prognostic significance in liver damage and fibrosis. Overt diabetes is predictive of advanced fibrosis and inflammation. However impaired glucose-tolerance is not. The advice on diet and exercise for six months after diagnosis may be a good strategy in NAFLD. The patients with normal-ALT without hepatomegaly, morbid-obesity and diabetes seem to have a good prognosis, however some of these patients may still require liver biopsy. (Acta gastroenterol. belg., **2007**, 70, **277-284**).

Key words : non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, serum transaminases, lipid peroxidation, insulin resistance.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease and the most frequent cause of incidentally discovered serum transaminase elevation in the general population (1-7). Many of NAFLD patients with incidentally discovered increased liver echogenicity in ultrasonographic examination (US) have normal serum transaminase levels (8,9). NAFLD is divided into two conditions, namely, non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL may transform to NASH (10). The correlation between imaging and histology is far from being satisfactory (11,12). Histological evaluation of the liver is indispensable for final diagnosis. The exact pathophysiological process that induces NASH is still unknown but many mechanisms at the cellular level have been proposed. In NASH, the vulnerable liver appears to be injured by reactive oxygen species (13-17). Therefore in this study, we investigated the potential association between oxidative stress, liver pathology, clinical and biochemical presentations in NASH. In previous studies, the range of alcohol intake of patients labeled as NAFLD was wide, ranging from 40 to 140 g per week (18,19). To overcome the confounding effects of even small amounts of alcohol, we selected non-drinkers. One hundred and five non-drinker subjects with biopsy proven NAFLD were prospectively studied.

Methods

Patients

One hundred and five patients with NAFLD were studied. The first presentation was with elevated transaminase levels, hepatomegaly or increased liver echogenicity in US. Most of those patients were referred from the internal medicine and endocrinology outpatient departments of the same hospital.

Inclusion criteria

Diagnosis was based on histology with exclusion of other etiologies such as alcohol intake (indeed all patients were teetotallers), hepatitis B, C, toxic and metabolic liver diseases etc. by history and relevant tests. Three patients with a history of hypothyroidism were included, because they were euthyroid at the time of enrollment.

Subjects with a history of recent potentially hepatotoxic drug intake were excluded. Informed consent was obtained from each of the participating subjects. All

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patients underwent US and computed tomographic (CT) examination.

Biochemical analysis

The laboratory evaluation included ALT, AST, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), bilirubin, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglyceride, serum ferritin, iron (Fe), Fe-binding capacity, and transferrin saturation (TS). These parameters were measured using the standard techniques of clinical chemistry laboratories.

For all non-diabetics, an oral glucose tolerance test (OGTT) was performed. Patients with normal fasting glucose, but with the levels of 140-200 mg/dl, 2 h after 75 g of glucose administration were considered as having impaired glucose tolerance. Fasting insulin levels was measured, and HOMA (homeostasis model assessment) insulin resistance (IR) index was calculated (IR % = glucose mg/dl / 18 × fasting insulin mU/ml / 22.5).

Biochemical parameters related to oxidative stress :

Nitric oxide (NO) levels were determined by the Griess reaction. A commercially available ELISA kit (R&D systems, Quantikine ; Wiesbaden-Nordenstadt , Germany) was used for this propose. Malondialdehyde (MDA) and superoxide dismutase (SOD) levels were measured in serum as well as in liver tissue homogenates. In addition, vitamin C and E levels were measured in sera and glutathione was measured in tissue homogenates. TNF-alpha receptor (TNF-sRp55) levels were also measured in sera as well. A fasting blood sample was collected on the day of liver biopsy for biochemical analysis. The liver biopsy tissue (2-3 mm) and serum specimens were stored at –80°C, until analysis, and processed within a month. MDA as an end product of fatty acid peroxidation was measured in liver homogenates and serum by the thiobarbituric acid reactivity assay as previously described (20). Serum and tissue Cu-Zn-SOD activities were determined by the method of Sun *et al.* (21) in which inhibition of nitro blue tetrazolium (NBT) reduction by Xanthine/Xanthin oxidase is used as a superoxide generator is provided. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. The total protein concentration was measured by the method of Lowry *et al.* (22). Tissue glutathione concentration was determined according to the method of Beutler *et al.* (23) using metaphosphoric acid for protein precipitation and 5'-5'-dithiobis-2-nitrobenzoic acid for color development. Plasma TNF-sRp55 concentrations were measured using commercially available human sTNFR-1 twin enzyme-linked immunosorbent assay (ELISA) kits with a sensitivity of 25 pg/mL at room temperature (HyCult Biotechnology ; Uden, The Netherlands). Plasma total ascorbic acid levels were assayed spectrophotometrically by the 2,4-dinitrophenylhydrazine method of Omaye *et al.* (24). Plasma vitamin E concentration was determined according to the methods of Quaife *et al.* (25).

Radiological studies

All US and CT examinations were performed by the same radiologist (S. O.) who was blinded to the clinical data. In US, increase of liver echogenity was graded from 0 to 3 : grade 0 : normal echogenity ; grade 1 : slight, diffuse increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders ; grade 2 : moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm ; grade 3 : marked increase in fine echoes with poor or nonvisualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver.

In non-contrast CT the liver-spleen density gradient (LSDG) was simply calculated by subtracting the density of the spleen from that of the right lobe of the liver (12).

Histological evaluation

Liver biopsies were fixed in AFA (Acetic acid, formaldehyde, ethanol) for 6-8 h and paraffin embedded liver biopsies were cut into minimum six slides including at least three sections per biopsy. Sections were stained with haematoxylin and eosin and for reticulum. Hepatic iron deposition was estimated according to Perls' staining Histological examination of liver biopsy specimens was done by the same pathologist (G. O.), who was blinded to the clinical data, using the evaluation system described by Brunt (26). The pathologist evaluated the biopsy material under the codes, and the codes were different in reference to scoring of steatosis, inflammation and fibrosis. Steatosis was graded from 1 to 3 based on the percent of hepatocytes in the biopsy involved (grade 1 is up to 33% ; grade 2 is 33-66% ; grade 3 is > 66%). We defined NAFL as hepatocellular steatosis without fibrosis and prominent inflammation, and NASH as steatosis, ballooning degeneration of hepatocytes, mixed acute and chronic lobular inflammation, and zone 3 perisinusoidal fibrosis. Inflammation was graded from 0 to 3. The severity of hepatic fibrosis (stage) was defined as follows : stage 1, zone 3 perisinusoidal/ pericellular fibrosis ; stage 2, zone 3 perisinusoidal / pericellular fibrosis with periportal fibrosis ; stage 3, zone 3 perisinusoidal/ pericellular fibrosis and portal fibrosis with bridging fibrosis ; stage 4, cirrhosis.

Statistical analysis

Differences among groups were assessed by using a two tailed-t test and chi square test. Analysis of variance (ANOVA) was used for comparisons between 2-3 subgroups. The significance of differences of continuous variables was calculated by repeated measures of ANOVA. Spearman rank correlation analysis was used

to identify the predictive parameters for histological severity. $P < 0.05$ was considered statistically significant.

Results

Characteristics of the patients

Fifty-four males and 51 females participated in the study. The mean age was 46.6 ± 9.7 years (range 22-69). Mean age and BMI of women were significantly higher in comparison to men (p = 0.0001 and 0.017 respectively). Mean transferrin saturation as well as serum ferritin levels were significantly higher in males (0.28 ± 0.08 vs. 0.20 ± 0.09, p = 0.0001, and 117.3 ± 75.7 vs. 71.1 ± 68.8 ng/ml, p = 0.002) and the mean serum iron level was significantly lower in females (70.93 ± 31.14 vs. 92.87 ± 30.91 mcg/dl, p = 0.001). Fatigue and/or right upper quadrant discomfort were the most common symptoms present in 48/105 (46%) of patients. To determine which clinical or laboratory parameters were predictive of histological severity, we entered age, BMI, diabetes, liver vertical span, AST, ALT, ALP, GGT, cholesterol, HDL, LDL, VLDL, triglyceride, ferritin, Fe, Fe-binding capacity, TS , free T3, free T4, TSH, in the statistical model. Presence of diabetes was independently associated with the presence of advanced fibrosis and inflammation (r = 0.33, p = 0.000 and r = 0.29, p = 0.002, respectively). Serum ferritin levels correlated with fibrosis stage (r = 0.35, p = 0.000) as well. Seven patients (4 male and 3 female) had advanced fibrosis (stage 3), and three patients (2 male and 1 female) had cirrhosis. The AST/ALT ratio was over 1.0 in 2 out of 3 cirrhotics and 3 out of 7 of patients with advanced fibrosis. Some characteristics of the patients studied are shown in table 1.

Patients with Diabetes mellitus

Thirty-one subjects (29.5%) were diabetic and the frequency of diabetes was found to increase with advancing age (p = 0.02). BMI of diabetics was higher in comparison to non-diabetics (p = 0.03) and inflammation as well as fibrosis was more severe in diabetics in comparison to others (p = 0.002 and 0.0001, respectively). Four patients (1 male and 3 female) had advanced fibrosis (stage 3), and 2 females had cirrhosis.

Comparison of patients with normal and elevated ALT levels

In 29 patients, the initial serum ALT level was in normal range, while in 76, it was above the upper range of normal. In comparing the group with normal ALT against the group with elevated ALT levels, plasma NO levels (p = 0.007) were significantly lower in the normal ALT group, while VLDL levels were higher (p = 0.011). Fibrosis in liver biopsy and liver tissue MDA levels were significantly higher in the elevated ALT group (p = 0.012 and p = 0.031). US grade was significantly higher and LSDG score in CT was significantly lower in patients with elevated versus normal ALT (p = 0.005 and 0.038, respectively). There was no difference in reference to any other variable studied. Comparative data of patients with normal and elevated ALT levels are presented in table 2, figure 1.

Table 1. — **Clinical characteristics of the patient population at enrollment**

Total number of patients	**n = 105 (%)**
Men/women	54/51 NS
Mean age (years) Men Women	46.6 ± 9.7 43.5 ± 9.2 49.9 ± 9.1 p = 0.0001
Mean BMI Men Women	29.9 ± 4.3 28.9 ± 3.7 30.9 ± 4.7 p = 0.017
Complaints : Fatigue Right upper quadrant discomfort	48 (46) 31(29.5) 42(40)
Risk factors BMI < 25 Overweight BMI : 26-29 Obesity BMI : 30-34 Morbid obesity BMI ≥ 35 Diabetes mellitus Hyper triglyceridemia Hyper cholesterolemia History of hypothyroidism	 12 (11.4) 47 (44.8) 31 (29.5) 15 (14.3) 31 (29.5) 42 (40.0) 68 (64.8) 3 (2.9)

Comparison of patients with NAFL to patients with NASH

The male/female ratio was 17 to 21 in the NAFL and 37 to 30 in the NASH group. The mean age and BMI of the groups were comparable. The mean age of females was significantly higher in the NASH group compared to that of the NAFL group (52.6 ± 8.6 and 46.1 ± 8.4, p = 0.009, respectively). The liver vertical span measured in the mid-clavicular line was near-significantly higher in NASH (7.5 ± 2.6 vs. 5.8 ± 2.7 cm, p = 0.051). There was no difference in reference to serum ALT, AST, GGT and ALP levels. However the number of patients whose initial ALT levels were in normal range were more prevalent in the NAFL group (15/38 vs. 14/67, p = 0.041). Serum cholesterol and LDL levels were higher in the NAFL group (231.6 ± 65.9 vs 209.5 ± 41.8 mg/dl, p = 0.038 and 146.3 ± 38.8 vs. 129.1 ± 34.6 mg/dl, p = 0.024). Serum ferritin levels were significantly higher in the NASH group (109.7 ± 81.5 vs. 71.2 ± 58.2 ng/ml, p = 0.016).

Comparing the NAFL versus NASH groups in reference to serum and tissue lipid peroxidation markers ; plasma NO and plasma MDA levels (p = 0.05, for both) were significantly higher, and plasma vitamin E and C levels were significantly lower in NASH patients (p =

Table 2. — **Comparative data of subjects with normal versus elevated ALT**

Variable	Normal ALT (n = 29)	Elevated ALT (n = 76)	p
Men/women	12/17	42/34	NS
Age (years)	48.4 ± 7.3	45.9 ± 10.4	NS
BMI (kg/m^2)	30.6 ± 5.4	29.6 ± 3.9	NS
Liver vertical span (cm)	6.3 ± 2.4	7.1 ± 2.8	NS
ALP (U/l) (NR : 38-155)	133.5 ± 82.9	144.9 ± 56.4	NS
GGT (U/l) (NR : 7-49)	46 ± 67.8	68 ± 51.9	NS
Cholesterol (mg/dl) (NR : 50-200) HDL LDL VLDL	214.8 ± 42.8 39.2 ± 8.2 131.0 ± 41.2 45.5 ± 29.4	218.5 ± 56.1 44.4 ± 14.8 137.3 ± 35.3 33.4 ± 16.8	NS NS NS **0.011**
Triglyceride (mg/dl) (NR : 50-200)	219.3 ± 91.9	188.8 ± 102.7	NS
Ferritin (ng/ml)	85.4 ± 73.2	99 ± 76.9	NS
Fe (mcg/dl) (NR : 50-167)	75.6 ± 33.1	85.6 ± 34.4	NS
Fe binding capacity (mcg/dl) (NR : 200-450)	315.4 ± 48.5	337.7 ± 53.4	0.06
Transferrin saturation (%)	0.24 ± 0.09	0.25 ± 0.09	NS
Plasma MDA (mmol/L)	3.7 ± 0.47	4.17 ± 0.76	NS
Plasma SOD (U/ml)	24.62 ± 1.19	25.03 ± 1.26	NS
Plasma TNFp (pg/ml)	2864.7 ± 207.84	3022.45 ± 403.43	NS
Tissue MDA (nmol/g. wet tissue)	42.24 ± 16.06	51.37 ± 6.97	**0.031**
Tissue SOD (U/mg. protein)	1.29 ± 0.38	1.35 ± 0.32	NS
Tissue GH (mmol/g. wet tissue)	1.98 ± 0.35	1.84 ± 0.49	NS
Plasma vitamin E (mg %)	0.9 ± 0.02	0.83 ± 0.14	NS
Plasma vitamin C (mg %)	1.15 ± 0.12	1.07 ± 0.13	NS
Plasma NO (mmol/L)	40.3 ± 6.63	48.77 ± 9.02	**0.007**
Histological steatosis grade	1.67 ± 0.77	1.93 ± 0.78	NS
Inflammation grade	1.17 ± 0.54	1.3 ± 0.73	NS
Fibrosis stage	0.6 ± 0.7	1.2 ± 1.1	**0.012**
US grade	1.06 ± 1.03	2.3 ± 0.9	**0.005**
Liver-spleen density gradient	2.15 ± 9.8	-11.9 ± 21.08	**0.038**

NR : Normal range.

0.002, and p = 0.001, respectively). Excluding patients with diabetes mellitus, the insulin resistance parameters were similar between the groups. The US grade was significantly higher and LSDG was significantly lower in NASH in comparing to NAFL (US : 1.9 ± 0.8 vs. 1.4 ± 1.0, and LSDG : -7.0 ± 15.3 vs. 2.8 ± 15.4, p = 0.005 for both). Comparative data of patients are shown in table 3.

Radiological findings

In 105 patients, US grading correlated well with the histological steatosis grade (r = 0.43, p = 0.0001), and fibrosis (r = 0.17, p = 0.015). Overall there was good correlation between US grading and LSDG in CT (r = -0.51, p = 0.0001). When we compared the results of US and CT examinations for patients with BMI > 35 kg/m^2, CT-examination had a significantly better correlation with the steatosis grade compared to US (p = 0.016).

Histopathological findings

All histological parameters correlated with each other significantly : steatosis with necroinflammation (r = 0.42, p = 0.0001), steatosis with fibrosis (r = 0.28, p = 0.003), and necroinflammation with fibrosis (r = 0.51, p = 0.0001). The histopathological hepatic iron stain did not show any iron deposition.

Correlations of liver oxidative stress related parameters

The increases in plasma MDA, SOD and NO levels were correlated with transferrin saturation (r = 0.50, p = 0.003 ; r = 0.47, p = 0.006, and r = 0.39, p = 0.026, respectively) and with high iron levels (r = 0.52, p = 0.002 ; r = 0.46, p = 0.008 ; r = 0.39, p = 0.026, respectively). There was an inverse correlation between serum iron levels and plasma vitamin E levels (r = -0.57, p =

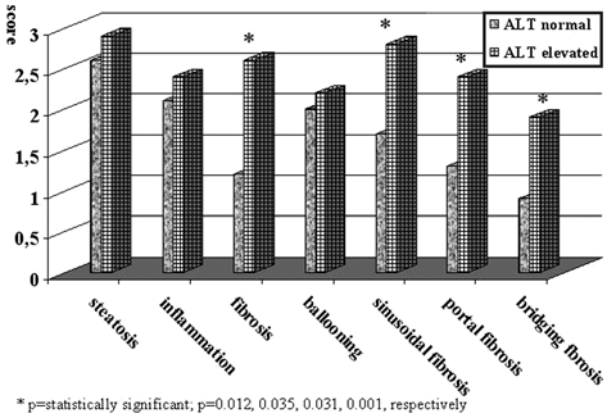

Fig. 1. — Histopathological comparison of normal versus elevated ALT in patients with non-alcoholic steatohepatitis. There was no difference with respect to the grade of steatosis and inflammation. Fibrosis stage, sinusoidal, portal, and bridging fibrosis were significantly higher in elevated ALT group (p = 0.027, 0.035, 0.031, and 0.001, respectively).

0.001), and between serum ferritin and vitamin C levels (r = -0.45, p = 0.01), respectively. Transferrin saturation was also inversely correlated with vitamin E and C levels (r = -0.60, p = 0.0001 and r = -0.44, p = 0.01). There was no relation between serum TNF-alpha receptor levels and other parameters.

Follow-up period

Patients were followed up with advice on diet and exercise for six months. Significant reductions in ALT and GGT levels were achieved in the NAFL group (from 73.9 ± 101.9 U/l to 38.8 ± 15.8 U/l, p = 0.007, and from 72.1 ± 74.7 U/l to 38.7 ± 27.8 U/l, p = 0.013, respectively) as well as in the NASH group (from 66.5 ± 38.4 U/l to 57.6 ± 39.7, p = 0.026, and from 58.7 ± 42.4 U/l to 46.2 ± 29.4, p = 0.006, respectively).

Within a six month period, the serum ALT levels decreased into normal range in 23 of 76 (30.3%) NAFLD patients with initially high ALT whereas it increased in 3 of 29 (10.3%) patients with initially normal values. In the group with initially elevated ALT normalization was achieved in 11 of 23 (47.8%) of NAFL patients, and in 12 of 53 (22.6%) NASH patients (p = 0.043).

There was a significantly higher weight reduction in patients who achieved normal ALT levels after three and six months (p = 0.001, for both). Serum ALT at 6th month correlated with the severity of steatosis (r = 0.27, p = 0.023), inflammation (r = 0.26, p = 0.035) and fibrosis (r = 0.31, p = 0.011) in initial biopsy.

Discussion

This study evaluates several aspects of NAFLD. These patients were mostly diagnosed by an elevated ALT. However, a previous study performed on 51 patients with normal ALT-NAFLD revealed that advanced fibrosis was present in 12, and, cirrhosis in another 6 patients. The reason for biopsy in these patients was hepatomegaly in 21 subjects. The mean age ± SD of the subjects was 53 ± 7, and 29 out of 51 were diabetic (27). We also found that the liver vertical span measured in mid-clavicular line was near significantly higher in the NASH group (p = 0.051). In our study, there was a significant association between elevated ALT levels and the diagnosis of NASH. Fibrosis was significantly higher in the elevated ALT group (p = 0.012). The number of patients whose initial ALT levels were in normal range were significantly more prevalent in the NAFL group compared to the NASH group (15/38 vs. 14/67,p = 0.041). In most of the subjects, ALT elevation was mild to moderate. However there were 3 patients whose initial ALT were over 10 times the upper-limit of normal and interestingly all were in the NAFL group. These extreme elevations affected the mean ALT of the NAFL patients to the degree that their mean ALT level was insignificantly higher in comparison to the NASH group.

In a cross-sectional study of 143 patients with NASH, Angulo *et al.* found that advanced age (> 45), AST/ALT levels grater than 1.0, obesity and type II diabetes mellitus were predictive of advanced fibrosis (17). In our study the AST/ALT ratio was over 1.0 in 2 out of 3 cirrhotics and 3 out of 7 patients with advanced fibrosis. All the females and one male had diabetes mellitus. The mean age of 5 females were significantly higher than 5 males (55.4 ± 9.2 vs. 37.4 ± 11.2, p = 0.02). The same was true in reference to transferrin saturation, this time higher in males. It may be speculated that as far as diabetes mellitus is not present, the risk for advanced fibrosis and cirrhosis is insignificant in females, probably due to the protective effect of low iron load. In a previous study it was shown that NAFLD is twice as common in post-menopausal women in comparison to pre-menopausal ones and hormone replacement treatment decreases NAFLD rate (18). All this evidence shows that female hormones may also be protective against appearance and progress of NAFLD. In males, progressive liver disease may develop at earlier ages even if diabetes mellitus is not present.

Presence of overt diabetes mellitus had a significant impact on the severity of inflammation and fibrosis in liver biopsy. The same cannot be said about impaired glucose tolerance. In most of the non-diabetics fasting insulin levels were in normal range and there was no difference between the NAFL group and the NASH groups in reference to IR-index (3.03 ± 2.20 and 3.87 ± 3.32, p = 0.36.)

We do not have a perfect imaging modality to diagnose or refute NAFLD. In a previous study, the sensitivity, specificity, positive predictive value and negative predictive value of US in the diagnosis of NAFLD were 67%, 77%, 77%, and 67% respectively (12). This gives an overall error rate of 25-33% (11). In a previous study,

Table 3. — **Comparative data of patients with NAFL versus NASH**

Variable	NAFL, n = 38	NASH, n = 67	p
Normal ALT	15	14	**0.041**
Elevated ALT	23	53	
Men/women	17/21	37/30	NS
Age (years)	45.9 ± 9.3	47.1 ± 9.9	NS
BMI (kg/m²)	30.1 ± 5.3	29.8 ± 3.7	NS
Liver vertical span (cm)	5.8 ± 2.7	7.5 ± 2.6	0.051
AST (U/L) (NR : 5-37)	70.3 ± 131.0	50.4 ± 33.7	NS
ALT (U/L) (NR : 5-37)	94.9 ± 135.1	70.3 ± 40.5	NS
ALP (U/L) (NR : 38-155)	140.7 ± 78.2	142.4 ± 55.9	NS
GGT (U/L) (NR : 7-49)	65.2 ± 67.7	60.4 ± 51.3	NS
Cholesterol (mg/dl) (NR : 50-200) HDL LDL VLDL	231.6 ± 65.9 43.6 ± 11.1 146.3 ± 38.8 33.3 ± 14.9	209.5 ± 41.8 42.5 ± 14.7 129.1 ± 34.6 39 ± 24.6	**0.038** NS **0.024** NS
Triglyceride (mg/dl) (NR : 50-200)	192.8 ± 106	199.9 ± 97.7	NS
Ferritin (ng/ml)	71.2 ± 58.2	109.7 ± 81.5	**0.016**
Fe (mcg/dl) (NR : 50-167)	80.3 ± 39.5	84.2 ± 28.4	NS
Fe binding capacity (mcg/dl) (NR : 200-450)	338.7 ± 55	327.3 ± 51.4	NS
Transferrin saturation (%)	0.23 ± 0.1	0.26 ± 0.08	NS
IR-Index (%)	3.03 ± 2.2	3.87 ± 3.32	NS
Plasma MDA (mmol/L)	3.9 ± 0.82	5.9 ± 0.6	**0.05**
Plasma SOD (U/ml)	24.9 ± 1.09	24.9 ± 1.4	NS
Plasma TNFr (pg/ml)	2975.9 ± 427.7	2971.5 ± 321.6	NS
Tissue MDA (nmol/g wet tissue)	46.9 ± 6.8	49.5 ± 13.3	NS
Tissue SOD (U/mg protein)	1.3 ± 0.4	1.3 ± 0.3	NS
Tissue GH (mmol/g wet tissue)	1.8 ± 0.5	1.9 ± 0.4	NS
Plasma vitamin E (mg %)	1.9 ± 0.2	0.8 ± 0.12	**0.002**
Plasma vitamin C (mg %)	2.5 ± 0.1	1.0 ± 0.1	**0.001**
Plasma NO (mmol/L)	44.75 ± 8.09	50.1 ± 9.8	**0.05**
US grade	1.4 ± 1.0	1.9 ± 0.8	**0.005**
Liver-spleen density gradient	2.8 ± 15.4	-7.0 ± 15.3	**0.005**

NR : Normal range.

no correlation was found between US and CT findings and the presence of NAFL or NASH (13). However our study showed that US grade correlated well with the histological steatosis grade ($p = 0.0001$), and fibrosis ($p = 0.015$). Reactive oxygen species (ROS) mediated liver injury has certainly an irrefutable role in the pathogenesis of NAFLD, and its presence may harbor transformation from NAFL to NASH (29-32). Failure of antioxidant defense mechanism with the depletion of glutathione and vitamin E may facilitate this transformation (33-35). Oxidative stress also stimulates the synthesis of several cytokines and the by-products of lipid peroxidation (hydroxyl radical, MDA) and induces stellate cell activation, fibrogenesis and direct hepatocyte damage (36-39). In our study, fibrosis, plasma NO levels as well as tissue MDA levels were significantly higher in elevated ALT group ($p = 0.012$, 0.007, and 0.031, respectively). In comparing NAFL patients to patients with NASH, plasma MDA, plasma NO and ferritin levels were significantly higher in NASH ($p = 0.05$, 0.05, and 0.016, respectively). On the other hand plasma vitamin E and C levels were significantly lower in the NASH group ($p = 0.002$, and 0.001, respectively). In addition plasma MDA, SOD, and NO levels correlated positively with high iron levels ($p = 0.002$, 0.008, and 0.026, respectively) and high transferrin saturation ($p = 0.003$, 0.006, and 0.026, respectively). This data point towards a highly significant role of ROS-mediated damage in the progression of NAFLD. It seems likely that serum ALT as well as ferritin levels do have some prognostic significance in reference to ongoing ROS-induced damage and fibrosis (34,36,39).

Serum ALT is a highly dynamic feature in NAFLD, mostly in a downward fashion after diagnosis and dietary advice. In the present study, while the ALT value normalized in 47.8% of the patients with NAFL, it occurred in only 22.6% of NASH patients. Three patients whose ALT levels were normal at initial testing but found to be high at the 6th month were determined to have NASH after histological evaluation. Because serum ALT levels show an undulant course in NAFLD, the interpretation of the data as initial high ALT or failure to normalize in 6 months points towards a more severe disease may be misleading.

In conclusion, it appears that the probability of severe liver disease is slightly higher in patients with elevated ALT in comparison to normal ALT in NAFLD. However, extreme elevations in ALT may be found in NAFL without hepatitis. ROS-induced liver damage may indeed be responsible in the transformation of NAFL to NASH. In addition, serum ferritin levels do have some prognostic significance in reference to ongoing ROS-induced damage and fibrosis (33-40).

Overt diabetes mellitus is predictive of advanced fibrosis and inflammation. However impaired glucose tolerance is not. Expectant management with advice on diet and exercise for six months after diagnosis may be a good strategy in NAFLD. The patients with normal-ALT without hepatomegaly, morbid-obesity and diabetes seem to have a good prognosis, however some of these patients may still require liver biopsy.

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