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Increased Orocecal Transit Time in Patients with Nonalcoholic Fatty Liver Disease

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Intestinal bacterial overgrowth (IBO) has been suggested to play a pathogenic role in patients with nonalcoholic fatty liver disease (NAFLD). Delayed intestinal transit may contribute to IBO development. Ten nondiabetic patients with NAFLD and abnormal liver enzymes were recruited. Ten healthy individuals, matched by sex, age, and body mass index, were used as controls. Orocecal transit time (OCTT) was measured by the lactulose breath test. Anti-endotoxin core antibodies (EndoCAb) were determined. The effect of oral norfloxacin (400 mg BID during 2 weeks) on liver enzymes, lactulose breath test, and EndoCAb was also studied. NAFLD patients had higher basal breathed H_2 and prolonged OCTT compared to controls (127 ± 61 vs. 57 ± 23 min, respectively; $P = 0.0037$). EndoCAb titers were similar in NAFLD patients and controls. Norfloxacin administration had no effect on ALT levels, lactulose breath test, or EndoCAb titers in patients with NAFLD. The present data show evidence of deranged intestinal motility in nondiabetic patients with NAFLD and support the hypothesis that NAFLD could be linked to endotoxin-induced liver damage of intestinal origin.

KEY WORDS: nonalcoholic fatty liver; steatohepatitis; fatty liver; intestinal bacterial overgrowth; anti-endotoxin antibodies; EndoCAb.

The term nonalcoholic fatty liver disease (NAFLD) encompasses several clinical-pathological entities (isolated steatosis, steatonecrosis, steatohepatitis, and histological alcoholic-like hepatitis) that are similar to those seen in alcoholic liver disease (1–3). When inflammation or perisinusoidal fibrosis is present on histological examination, the term nonalcoholic steatohepatitis (NASH) is used following Brunt criteria (4). The exact prevalence of NAFLD is unclear, but it is becoming evident that the disease is much more common than previously thought (5). Although generally a benign, indolent process, it can lead to liver fibrosis in up to a third of patients (1). Several metabolic factors are clearly associated with the development of liver steatosis, particularly insulin resistance, which seems to play a central pathogenic role (1, 6).

There is ongoing debate about the factors that can trigger and maintain inflammation in NASH. Intestinal bacterial overgrowth (IBO) is one of them and has been suggested to play a pathogenetic role through low-grade endotoxemia (7, 8). The association between small intestinal diverticulosis and jejuno-ileal bypass with NASH and severe liver damage including cirrhosis (9, 10) are two interesting clinical observations that give support to this hypothesis. Recent studies performed in patients with NASH have suggested that these patients have a higher prevalence of IBO, as assessed by the ^{14}C-D-xylose-lactulose breath test, and higher TNF-α levels in comparison with control subjects (8). The mechanism responsible of the occurrence of IBO in NASH patients is not clear. Considering that a significant proportion of these patients are diabetic, it is conceivable that the intestinal transit time could be

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delayed thus promoting the development of IBO. In this study, we measured orocecal transit time (OCTT) in nondiabetic NAFLD patients with abnormal liver enzymes using lactulose breath test (LBT) and assessed markers of endotoxin exposure with the hypothesis that a prolonged OCTT may contribute to IBO in NAFLD patients. In addition, we assessed the effect of short-term administration of nonabsorbable antibiotics on liver function tests and OCTT and IBO in these patients.

PATIENTS AND METHODS

Ten patients with NAFLD and elevated liver enzymes seen in our outpatient liver clinic were recruited. They fulfilled the following criteria: (a) abnormal ALT for at least 6 months; (b) alcohol intake of <10 g/day, assessed independently by a physician and a nurse; (c) abdominal ultrasound showing diffuse increase in liver echogenicity; (d) absence of other causes of chronic hepatitis (negative results for anti-HCV, HBsAg, total anti-HBc, antinuclear antibodies, anti-smooth muscle antibodies, and additional tests when clinically suspected); (e) absence of clinical or histological evidence of cirrhosis; (f) absence of anatomical conditions promoting intestinal stasis or recirculation of enteric contents, such as small intestine diverticula, surgical blind loops, and fistula; and (g) absence of conditions associated with impaired intestinal motility, including diabetes mellitus, scleroderma, and intestinal pseudo-obstruction. Ten healthy individuals, matched by sex, age, and body mass index, were used as controls. Percutaneous liver biopsy was proposed to all patients.

LBT was performed using a standard protocol (11) in all individuals. Briefly, patients were asked to stop smoking and follow a diet low in complex carbohydrates the day before the test. The day of the test, subjects were fasting and lactulose test was performed measuring H_2 (Hoek Loos Lactoscreen) exhaled at time 0 and every 15 min since the ingestion of 10 g of lactulose (100 mL of a 10% solution). Measurements were continued up to the moment when the subject reached an increment of 10 parts per million (ppm) or greater compared to the basal value of exhaled H_2 or for at least 90 min. OCTT was calculated from the instant of the administration of lactulose to the moment when it reached an elevation greater than 10 ppm from the basal value in two consecutive determinations (11, 12). Serum aminotranferases, glucose, cholesterol, and triglycerides were determined using standard methods (automatic analyzer Modular Hitachi).

In addition to basal determinations, patients with NAFLD received a 14-day course of norfloxacin at a dose of 400 mg BID. Lactulose breath test and serum ALT were then repeated.

Serum samples for measurement of IgG and IgM endotoxin core antibodies (EndoCAb; Eskia Inc., Edinburgh, Scotland) were obtained at baseline in all subjects and after treatment in NAFLD patients. Measurement of EndoCAb was performed in duplicate according to the instructions supplied by the manufacturer (13). Results of determinations are expressed as standard median units per milliliter (GMU/mL and MMU/mL for IgG and IgM, respectively).

The study was approved by the Institutional Ethics Committee. All individuals signed a written informed consent and voluntarily participated in the study.

Statistical analysis was performed using the Instat software version 3.0 (GraphPad Software, Inc). Mann–Whitney and paired and unpaired *t* tests were used as appropriate to compare proportions and means.

TABLE 1. DEMOGRAPHIC AND LABORATORY DATA (MEAN ± SD) OF NAFLD PATIENTS AND CONTROL SUBJECTS

Characteristic	*Patients*	*Controls*	P *value*
N	10	10	—
Sex (male/female)	2/8	2/8	NS
Age (years)	48.4 ± 8	50 ± 7.6	NS
Weight (kg)	74.6 ± 9.3	76.5 ± 15	NS
Height (cm)	154.8 ± 7.1	170.6 ± 7.2	NS
BMI (kg/m^2)	31.2 ± 4.5	29.7 ± 6.6	NS
Cholesterol (mg/dL)	203 ± 29	238 ± 43	0.0458
Triglycerides (mg/dL)	181 ± 87	194 ± 194	NS
Glucose (mg/dL)	95 ± 12	92 ± 12	NS
ALT (UI/L)	49.5 ± 34	28.7 ± 23	NS
AST (UI/L)	69.3 ± 50	29.8 ± 14	0.0258

RESULTS

Demographic and laboratory data on patients and controls are shown in Table 1. The groups were comparable in all aspects, except in cholesterol and AST serum levels. Mean cholesterol levels were 203 ± 29 mg/dL in patients and 238 ± 43 mg/dL in controls ($P = 0.046$), while AST levels were 69.3 ± 50 UI/L in patients and 29.8 ± 14 UI/L in controls ($P = 0.026$). Liver biopsy was performed in five patients. All of them had steatohepatitis on histological examination with diverse stages of fibrosis, but none of them had cirrhosis.

Mean basal value of breathed H_2 in NAFLD patients was 10.8 ± 3.3 ppm, vs. 6.3 ± 3.3 ppm in controls ($P = 0.0084$). Statistically significant differences were also found between patients and controls in breathed H_2 at 15 and 105 min after lactulose administration (Figure 1).

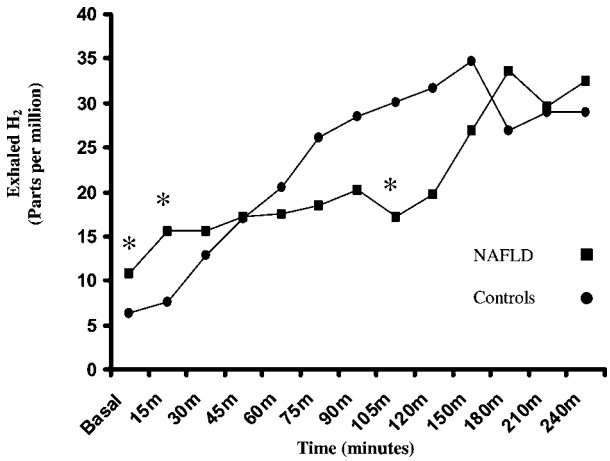

Fig 1. Lactulose breath test in NAFLD patients and controls. Mean values of breathed H_2 are depicted over 240 min. Asterisks denote statistically significant differences between groups ($P < 0.05$).

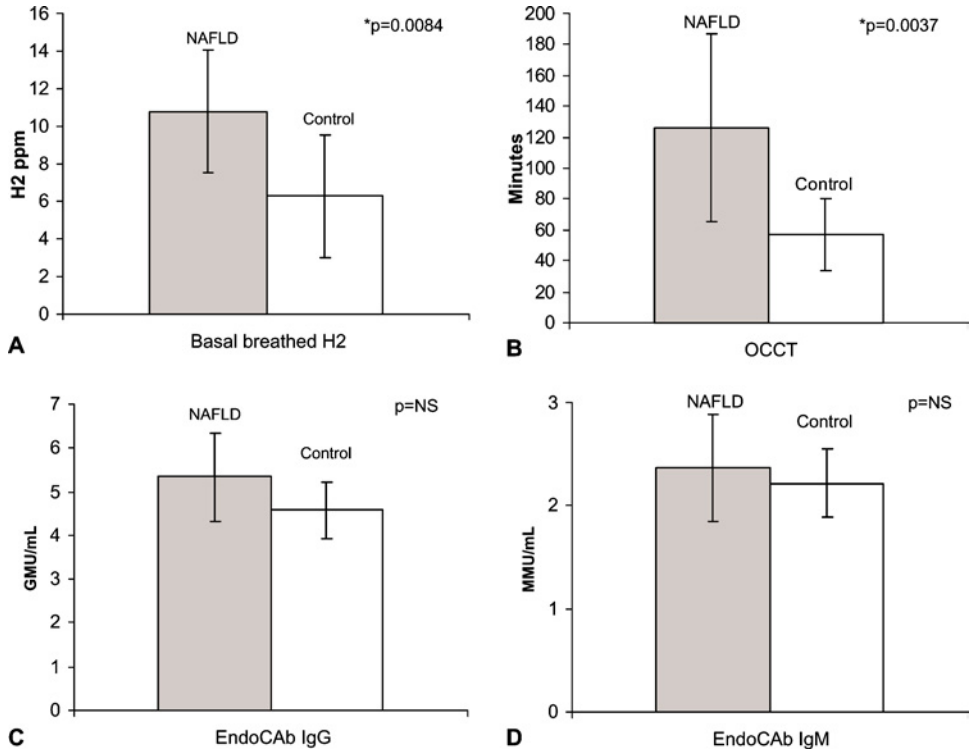

Fig 2. Comparison of NAFLD patients with controls. (A) Basal breathed H_2. (B) Orocecal transit time. (C) EndoCAb titers (IgG). (D) EndoCAb titers (IgM).

Sixty patients with NAFLD had a breathed H_2 concentration $\geq$12, but none of the controls did. OCTT was significantly prolonged in patients with NAFLD (127 $\pm$ 61 min) compared to control subjects (57 $\pm$ 23 min; $P = 0.0037$) (Figure 1).

Higher levels of anti-endotoxin antibodies were found in NAFLD patients than controls, but differences were not statistically significant: mean EndoCAb IgG level was 5.34 GMU/mL in NAFLD patients and 4.58 GMU/mL in controls ($P = 0.1$); mean EndoCAb IgM was 2.37 and 2.22 MMU/mL, respectively ($P = 0.3$) (Figure 2).

Short-term norfloxacin administration was well tolerated. All patients completed the treatment period with no adverse events. Treatment with norfloxacin for 14 days had no effect on lactulose breath test results, ALT levels, or EndoCAb titers in patients with NAFLD (data not shown).

DISCUSSION

NAFLD is one of the most common causes of liver disease in Western countries, with the potential to progress to advanced liver fibrosis and cirrhosis (6). Unfortunately there are no approved treatments for this disease. Pathogenic mechanisms are only partially known and the relative importance of different factors associated with the disease is unclear. NAFLD is commonly viewed as a two-stage process in which insulin resistance leads to steatosis in hepatocytes ("first hit") (14). Several different mechanisms have been implicated as the elusive "second hit." Increased oxidative stress seems to be common to all the mechanisms implicated in this step. Clinical observations suggest that liver inflammation could be related to exposure to endotoxins of intestinal origin (9, 10).

The purpose of this study was to determine if NAFLD patients had altered intestinal motility or evidence of IBO. Breath tests have recently been claimed to be insensitive for assessing IBO (7). Indeed, the most accurate procedure for confirming presence of IBO is the bacteriological analysis of the jejunal aspirate (15). However, the latter method requires intestinal intubation and is not generally well tolerated. The lactulose breath test has been used as an indirect indicator of IBO in several settings such as alcoholic and nonalcoholic cirrhosis (16). In the present study, we found that 6 of 10 patients had evidence of IBO defined as high basal H_2 excretion, but none of the controls. Recently Wigg *et al.* (8) published data concerning the prevalence of IBO, intestinal permeability, endotoxemia, and TNF-α in patients with NASH. Using the lactulose breath test they found a greater prevalence of IBO

in NASH patients. However, selection of patients in that study may be a confounding factor because they enrolled patients with diabetes mellitus. In addition, NASH patients had significantly higher BMIs than controls. Diabetes and obesity are both related to developing IBO (17, 18). For these reasons, in the present study we excluded patients with diabetes mellitus and carefully matched BMI between patients and controls. We also matched patients by age, since it has been reported that elderly patients have prolonged OCCTs compared with young people (19). In spite of these cautionary measures, our results are in agreement with data of Wigg *et al.* supporting the occurrence of IBO in NASH patients.

In addition to evidence of IBO, we observed prolonged OCTTs in NAFLD patients compared to controls, which suggests deranged intestinal transit in these subjects. It is not clear if alteration of intestinal motility is a cause or a consequence of IBO. In fact several studies have shown that administration of prokinetic drugs such as cisapride may indeed improve IBO in experimental and clinical cirrhosis (20, 21), while short-term antibiotics improve prolonged OCCT in diabetic patients (17). In the present study, norfloxacin administration had no effect on IBO or OTTC in patients with NAFLD. Furthermore, antibiotic treatment did not normalize aminotransferase levels. The lack of efficacy of a 2-week course of norfloxacin in normalizing ALT may be related to insufficient dose or time and also raises the question of whether different approaches like using polymixin, which has endotoxing-binding properties, or alternative ways of modifying the intestinal flora (probiotics) would modify the natural history of NASH.

The present study supports that patients with NAFLD and elevated liver enzymes have evidence of IBO as reflected in the higher basal and early breathed H_2 values in the lactulose breath test. Moreover, our data show that patients with NAFLD have evidence of slower intestinal transit time assessed by the lactulose test. Thus, it may be hypothesized that an altered intestinal motility may be related to the occurrence of IBO and in turn may be related to endotoxin-induced liver inflammation in patients with NAFLD as seen in patients with small intestinal diverticulosis (9) and jejuno-ileal bypass (10). Our findings, however, have to be interpreted cautiously and do not necessarily imply that prolonged OCCT is followed by IBO in NAFLD patients, since antibiotic treatment had no clinical effect and serum anti-endotoxin antibodies (EndoCAb), an indicator of endotoxin exposure, were similar in NAFLD patients compared to controls. Levels of EndoCab were measured in peripheral blood and may not necessarily be an expression of endotoxin levels in portal blood. In addition, it is possible that endotoxins not detected by EndoCab (for example, peptidoglycan polysaccharide [22]) are implicated in liver damage and that intestinally derived factors other than endotoxin may be contributing to NASH pathogenesis in patients with intestinal bacterial overgrowth.

There are no accepted effective medical interventions capable of modifying the natural history of NAFLD. The present data, if confirmed, may open new possibilities for the treatment of patients with this disease, either by modifying IBO or by restoring intestinal motility. The latter approach has been explored in cirrhotic patients to prevent bacterial translocation (21) and in experimental models of NASH with the use of probiotics (23).

In conclusion, present data show evidence of IBO and deranged intestinal motility in NAFLD patients with elevated serum aminotransferases levels, with no correction after treatment with nonabsorbable antibiotics. Whether or not these abnormalities are related to endotoxin-induced liver damage remains unclear at this time, providing a subject for further studies.

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