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Intestinal permeability is increased in children with non-alcoholic fatty liver disease, and correlates with liver disease severity

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ABSTRACT

Background: Increased intestinal permeability seems to play a major role in non-alcoholic liver disease development and progression.
Aim: To investigate the prevalence of altered intestinal permeability in children with non-alcoholic fatty liver disease, and to study its potential association with the stage of liver disease.
Methods: We performed a case–control study examining intestinal permeability in children using the lactulose–mannitol bowel permeability test.
Results: Overall, 39 consecutive patients (30 males, median age 12 years) and 21 controls (14 males, median age 11.8 years) were included. The lactulose/mannitol ratio resulted impaired in 12/39 patients (31%) and none of the controls. Intestinal permeability was higher in children with non-alcoholic fatty liver disease (lactulose/mannitol ratios: 0.038 ± 0.037 vs. 0.008 ± 0.007, $p < 0.05$). Within the non-alcoholic fatty liver disease group, intestinal permeability was increased in children with steatohepatitis compared to those with steatosis only (0.05 ± 0.04 vs. 0.03 vs. 0.03, $p < 0.05$). Pathological lactulose/mannitol ratio correlated with portal inflammation ($p = 0.02$), fibrosis ($p = 0.0002$), and ballooning of hepatocytes ($p = 0.003$). Blood lipopolysaccharides levels were higher in children with steatohepatitis (2.27 ± 0.68 vs. 2.80 ± 0.35, $p < 0.05$).
Conclusions: Intestinal permeability is increased in children with non-alcoholic fatty liver disease, and correlates with the severity of the disease.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is nowadays one of the most common chronic liver diseases, both in adults and children. The disease ranges from simple fat accumulation in the liver (steatosis) to inflammation and fibrosis, i.e. non-alcoholic steatohepatitis (NASH). The progression of NAFLD to the more severe form, NASH, is linked to both genetic and environmental factors [1]. Plenty of research focused on the interaction between the liver and the gut, since the so-called gut-liver axis appears to play a major role among the factors leading to disease progression [2,3]. In vitro and animal models of NAFLD showed that alteration of gut microbiota and increased intestinal permeability augment the exposure of the liver to gut-derived bacterial products, such as lipopolysaccharides (LPS). Increased serum levels of LPS and other intestinal bacterial products determine endotoxemia, especially in the portal system and the liver. Endotoxemia stimulates innate immune receptors, namely Toll-like receptors (TLR), which activate signalling pathways involved in liver inflammation and fibrogenesis [4]. Miele et al. [5] demonstrated that also in humans NAFLD is associated with increased intestinal permeability and small intestinal bacterial overgrowth (SIBO), and that these intestinal factors are associated with the severity of hepatic steatosis. Specifically, a progressive disruption of the tight junctions (TJ) was demonstrated in NAFLD patients, a process that might explain the contribution of intestinal products to liver disease progression.

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Intestinal permeability (IP) is a property of the intestinal epithelium allowing molecules to pass through by non-mediated diffusion. The transport of molecules across the intestinal epithelium takes place through 2 major routes, i.e. the transcellular and paracellular. Determining the urinary excretion of disaccharides and monosaccharides and the ratio of their excretion represents a method for the measurement of intestinal permeability. Monosaccharides, such as mannitol (M), pass through the transcellular routes of the aqueous pores, reflecting the degree of absorption of small molecules. Disaccharides, such as lactulose (L), pass through the intercellular junction complex, reflecting the permeability of large molecules. The lactulose and mannitol urinary excretion ratio is the most commonly used test for the assessment of small intestinal permeability [6,7].

The current study was designed to investigate the prevalence of altered IP in children with biopsy-confirmed NAFLD, and to study its potential association with the stage of liver disease and the development of systemic endotoxemia.

2. Methods

The protocol for this observational, non-interventional study was approved by the Ethic Committees of the Bambino Gesù Children Hospital and Sant'Andrea Hospital. The parents of all participants provided written informed consent to the tests performed and to the publication of the results.

2.1. Patients and controls

The study population consisted of paediatric patients with biopsy-proven NAFLD or NASH who were consecutively seen at the outpatient Hepato-metabolic Unit of the Bambino Gesù Children Hospital for chronically (at least 6 months) elevated aminotransferase levels of unknown origin and ultrasound evidence of hepatic steatosis. Exclusion criteria included evidence of any of the following: hypothyroidism, Wilson's disease, viral hepatitis (HBV, HCV), acute systemic disease, cystic fibrosis, coeliac disease (CD), irritable bowel syndrome (IBS) or other gastrointestinal functional disorders, suspicion of muscular dystrophy, alpha-1-antitrypsin deficiency, metabolic diseases, autoimmune hepatitis, drug toxicity, drugs known to induce steatosis (e.g. valproate, amiodarone, or prednisone) or to affect body weight and carbohydrate metabolism, parenteral nutrition, protein malnutrition, previous gastrointestinal surgery, structural abnormalities of the gastrointestinal tract, neurological impairment. Finally, the use of non-steroidal anti-inflammatory drugs, antibiotics, probiotics, or antisecretory drugs capable of causing achlorhydria within the 2 months preceding enrolment, was considered among the exclusion criteria.

For comparative purposes, we enrolled a group of control subjects, matched for gender, age, and ethnic origin (white) with those of the study group. The group was composed of normal-weight children ($N = 21$) referred to the Paediatric Unit of the Sant'Andrea Children Hospital for asthma ($N = 13$), upper respiratory infections ($N = 4$), and headache ($N = 4$), who had normal serum aminotransferase levels, no evidence of a "bright liver" on hepatic ultrasound examination, no history of functional and/or organic gastrointestinal disorder, and no family history of diabetes, celiac disease (CD), or dyslipidemia.

Complete medical histories and the results of the physical examination were recorded for all participants. Any complaint of gastrointestinal symptoms, such as bloating, diarrhoea, nausea, or vomiting, determined the exclusion from the study. The degree of insulin resistance/sensitivity was estimated with the homeostatic model assessment equation, as follows: (fasting insulin × fasting glucose)/405 [8], and with the Insulin Sensitivity Index equation, as follows: [10 000/sqrt (fasting glucose × fasting insulin × post-oral glucose at 120 min × post-oral insulin at 120 min) [9]. Body mass index (BMI) was calculated as patient's weight in kg divided by patient height (in metres) squared. Patients with BMI >85th percentile according to CDC charts [10] were considered obese.

2.2. Liver biopsy

The clinical indication for biopsy was to assess the presence of liver steatosis, NASH, and the degree of fibrosis or other likely independent or competing liver diseases. A liver biopsy was performed in all children included in the study, after an overnight fast, using an automatic core biopsy 18 gauge needle (Biopince, Amedic, Sweden) under general anaesthesia and ultrasound guidance. A Sonoline Omnia ultrasound machine (Siemens, Munich, Germany) equipped with a 5-MHz probe (5.0 C 50, Siemens) and a biopsy adaptor were employed. The length of the liver specimen was recorded: only samples with a length > 15 mm and including at least 6 complete portal tracts were considered adequate for the purpose of the study. Biopsies were routinely processed (i.e. formalin-fixed and paraffin-embedded) and sections of liver tissue were stained with haematoxylin–eosin, Van Gieson, Periodic acid-Schiff diastase, and Prussian blue stain. Biopsies were evaluated by a single hepatopathologist who was blinded to clinical and laboratory data. Steatosis, inflammation, hepatocyte ballooning, and fibrosis were scored using the NAFLD Clinical Research Network (CRN) criteria [11]. Steatosis was graded on a 4-point scale: grade 0 = steatosis involving <5% of hepatocytes; grade 1 = steatosis involving up to 33% of hepatocytes; grade 2 = steatosis involving 33%-66% of hepatocytes; and grade 3 = steatosis involving >66% of hepatocytes. Lobular inflammation was graded on a 4-point scale: grade 0 = no foci; grade 1 = <2 foci per 200× field; grade 2 = 2–4 foci per 200× field; and grade 3 = >4 foci per 200× field. Hepatocyte ballooning was graded from 0 to 2: 0 = none; 1 = few balloon cells; and 2 = many/prominent balloon cells. The stage of fibrosis was quantified using a 5-point scale: stage 0 = no fibrosis; stage 1 = perisinusoidal or periportal (1a = mild, zone 3, perisinusoidal; 1b = moderate, zone 3, perisinusoidal; 1c = portal/periportal); stage 2 = perisinusoidal and portal/periportal; stage 3 = bridging fibrosis; and stage 4 = cirrhosis. Features of steatosis, lobular inflammation, and hepatocyte ballooning were combined to obtain the NAFLD activity score (NAS). As recently recommended by NASH CRN [12], a microscopic diagnosis based on overall injury pattern (steatosis, hepatocyte ballooning, inflammation) as well as the presence of additional lesions (e.g. zonality of lesions, portal inflammation and fibrosis) was assigned to each case. Accordingly, biopsies were subdivided into: not-NASH (NAFLD) and definite NASH subcategories.

2.3. Intestinal permeability test: lactulose and mannitol ratio

Children underwent bowel permeability test with lactulose and mannitol. The selected patients followed a lactulose and mannitol-free diet for 24 h before the test. After an overnight fast, they voided a pre-test urine sample and ingested a solution containing 5 g of lactulose and 1 g of mannitol in 120 mL of deionised water. Urine was collected during the next 6 h, with 1 mL of chlorhexidine (1 mg/mL) added as preservative. One hour after the test was started, patients were encouraged to drink 50–150 mL of tap water. Total urine volume was measured, and a 10-mL aliquot was stored at −20 °C until analysis. The fractional excretion of lactulose was calculated from the following ratio: mg lactulose excreted/mg lactulose assumed. The mg of lactulose excreted was obtained from the equation: mg/L lactulose × litres of urine. The same calculation was performed for mannitol. The values of lactulose and mannitol calculated in the pre-test urine as mg/L were subtracted from the same value obtained in the urine collected during the 6-h test.