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Relationship between portal chronic inflammation and disease severity in paediatric non-alcoholic fatty liver disease

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ABSTRACT

Background: The non-alcoholic steato-hepatitis Clinical Research Network has recently shown that portal chronic inflammation is associated with liver fibrosis in American children with non-alcoholic fatty liver disease.
Aim: We tested whether the portal chronic inflammation-fibrosis association was present in a series of Italian children with non-alcoholic fatty liver disease.
Methods: We re-assessed the liver biopsies of 144 consecutive Italian children with non-alcoholic fatty liver disease aged 3–18 years and followed at the "Bambino Gesù" Paediatric Hospital. Non-alcoholic fatty liver disease and portal chronic inflammation were diagnosed using the non-alcoholic steato-hepatitis Clinical Research Network criteria. Anthropometry, body composition, liver enzymes, metabolic parameters and blood pressure were measured in all children.
Results: Two children had no portal chronic inflammation, 84 had mild and 58 more than mild portal chronic inflammation according to the non-alcoholic steato-hepatitis Clinical Research Network criteria. Children with no or mild portal chronic inflammation had the same clinical features of those with more than mild portal chronic inflammation except for insulin resistance, which was greater. There was no association between steatosis, lobular inflammation, ballooning, fibrosis and portal chronic inflammation.
Conclusion: We were not able to confirm the existence of a clinico-pathological association between portal chronic inflammation and disease severity in a series of Italian children with non-alcoholic fatty liver disease. Some clinico-pathological correlates of paediatric non-alcoholic fatty liver disease may be population-specific.

1. Introduction

Owing to the current epidemic of obesity and diabetes, non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease in both children and adults [1]. NAFLD affects 3% to 10% of children and adolescents, and this figure increases up to about 80% among obese individuals [2]. Because NAFLD may progress to non-alcoholic steato-hepatitis (NASH) and cirrhosis [3], its early identification in children and adolescents is important to prevent the development of chronic liver disease in later life.

Three forms of paediatric NASH have been identified so far: type 1, type 2 and overlap NASH [4,5]. Type 1 NASH is characterized by steatosis, ballooning and/or perisinusoidal fibrosis without portal inflammation; type 2 NASH by steatosis, portal inflammation and/or fibrosis without ballooning and perisinusoidal fibrosis; lastly, overlap NASH has features of both type 1 and type 2 NASH. In contrast with the frequently mixed inflammatory infiltrates that may occur in the lobules of NAFLD, portal inflammation is characterized by "chronic cells", i.e. lymphocytes, plasma cells, occasional eosinophils and monocytes [6,7]. It has been suggested that portal chronic inflammation (PCI) may be a harbinger of more serious concurrent liver disease and the NASH Clinical Research Network (CRN) has recently tested the hypothesis that PCI has clinico-pathological correlates in both adults and children [6]. In 205 children, the NASH CRN detected an association of PCI with fibrosis but not with body mass index (BMI) and insulin resistance [6].

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The present study aimed at testing whether these associations could be detected also in an external well-studied clinical series of Italian children and adolescents with NAFLD [8–11].

2. Subjects and methods

2.1. Study design

Using the NASH CRN criteria for PCI [6], we re-assessed the liver biopsies of 144 consecutive paediatric patients with NAFLD followed at the Liver Unit of the "Bambino Gesù" Paediatric Hospital between June 2005 and January 2009. Inclusion criteria were persistently elevated serum aminotransferase levels, diffusely hyperechogenic liver at ultrasonography and a liver biopsy diagnostic of NAFLD [11]. Exclusion criteria were alcohol consumption, hepatitis A, B, C, D, E or G, cytomegalovirus or Epstein-Barr virus infection, history of parenteral nutrition, use of drugs known to induce steatosis, autoimmune liver disease, celiac disease, Wilson's disease and α-1-antitrypsin deficiency. The study was approved by the Ethics Committee of the "Bambino Gesù" Paediatric Hospital and informed consent was obtained from the children or at least one responsible guardian.

2.2. Clinical and laboratory assessment

Weight and height were measured using standard procedures [12]. BMI was calculated and converted to standard deviation scores (SDS) using US reference data [13]. A standard deviation score indicates how many standard deviations an observation is above or below the mean and is obtained by subtracting the population mean from an individual value and then dividing the difference by the population standard deviation. Waist circumference was measured at the highest point of the iliac crest [11]. Percent body fat was measured by dual-energy X-ray absorptiometry using a QDR-1500 densitometer (Hologic Inc., Waltmann, MA, US) [11]. Alanine transaminase, aspartate transaminase, gamma-glutamyl-transferase, glucose, triglycerides and cholesterol were evaluated using standard laboratory methods. Insulin was measured by radio-immuno-assay (Myria Technogenetics, Milan, Italy). Glucose and insulin were measured at 0, 30, 60, 90 and 120 min during an oral glucose tolerance test performed with 1.75 g glucose per kg of body weight (up to 75 g) [11]. The homeostasis-model assessment index of insulin resistance (HOMA-IR) was calculated as [fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5] [14] and the insulin sensitivity index (ISI) as $10,000/\sqrt{[\text{fasting glucose (mg/dL)} \times \text{fasting insulin (μU/mL)} \times \text{mean glucose (mg/dL)} \times \text{mean insulin (μU/mL)}]}$ [15]. Blood pressure was measured as described in detail elsewhere [11]. The SDS of systolic and diastolic blood pressure were calculated from the reference data provided by the Fourth Report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents [16].

2.3. Liver histopathology

Liver biopsies were performed as described in detail elsewhere [11] and were scored using the NASH CRN criteria [6,17] by an experienced pathologist (RD) who was specifically trained to this aim at the Pathology Department, Washington University, Saint Louis, US (Director: Dr. E.M. Brunt). Steatosis was classified as 1 = 5–33%, 2 = 33–66%, 3 = >66%; lobular inflammation as 0 = no foci, 1 = <2 foci/200×, 2 = 2–4 foci/200×, 3 = >4 foci/200×; ballooning as 0 = none, 1 = few cells, 2 = many cells; fibrosis as 0 = none, 1 = perisinusoidal or periportal, 2 = perisinusoidal and portal/periportal, 3 = bridging; and PCI as 0 = none, 1 = mild, 2 = more than mild [6,17]. The NAFLD activity score (NAS) was calculated and a NAS ≥5 was considered to indicate NASH [17].

2.4. Statistical analysis

Continuous variables are given as median, interquartile range (IQR) and minimum and maximum values because of skewed distributions. IQR was calculated as the difference between the 75th and 25th percentile. Between-group comparisons of continuous variables were performed with an exact Mann–Whitney test and those of categorical variables with Fisher's exact test. The relationship between steatosis, lobular inflammation, ballooning, fibrosis and PCI was evaluated using an exact ordinal logistic regression continuation-ratio model [18,19]. Odds ratios (OR) and 95% exact confidence interval are reported. The OR obtained from the employed regression model is a measure of the change in the odds from less severe to more severe liver steatosis. All statistical tests were two-tailed and produced exact *p*-values. Statistical significance was set to a *p*-value <0.05. Statistical analysis was performed using Stata 11 (StataCorp, College Station, Texas, USA) and StatXact and LogXact 8.0 (Cytel Inc., Cambridge, MA, USA).

3. Results

We re-assessed the liver biopsies of 144 consecutive children and adolescents with NAFLD. These children had a median (IQR) age of 12 [4] years (range: 3–18 years) and were mostly males (68%, *n* = 98).

Steatosis was mostly 33–66% (40%), lobular inflammation <2 foci/200× (71%), ballooning none (53%), and fibrosis stage 1 (60%). PCI was mostly mild (58%) but a relevant proportion of children had a more than mild score (39%) (Table 1).

73% of the children with no or mild PCI were males (*n* = 63) as compared to 60% (*n* = 35) of those with more than mild PCI (*p* = 0.144). Because there were just two patients with no PCI, we collapsed them with those with mild PCI (*n* = 84) and compared this group to that with more than mild PCI (*n* = 58) (Table 2). Age, anthropometry, percent body fat, liver enzymes, blood lipids and blood pressure were similar in the two groups.

Table 1
Liver histopathology of 144 paediatric study patients.

	0		1		2		3		4	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Steatosis (degree)	–	–	39	27.08	58	40.28	47	32.64	–	–
Lobular inflammation (degree)	14	9.72	102	70.83	25	17.36	3	2.08	–	–
Ballooning (degree)	76	52.78	28	19.44	40	27.78	–	–	–	–
Fibrosis (stage)	41	28.47	87	60.42	5	3.47	11	7.64	–	–
Portal chronic inflammation (degree)	2	1.39	84	58.33	56	38.89	–	–	–	–