Digestive and Liver Disease 43 (2011) 143–146

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

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

Anna Alisi[a,1], Giorgio Bedogni[b,c,1], Rita De Vito[d], Donatella Comparcola[a], Melania Manco[e], Valerio Nobili[a,*]

[a] Liver Unit, Pediatric Hospital "Bambino Gesù", IRCCS, Rome, Italy
[b] Clinical Epidemiology Unit, Liver Research Center, Basovizza, Trieste, Italy
[c] Department of Maternal and Pediatric Sciences, University of Milan, Fondazione IRCCS Cà Granda - Ospedale Maggiore Policlinico, Milan, Italy
[d] Laboratory of Pathology, Pediatric Hospital "Bambino Gesù", IRCCS, Rome, Italy
[e] Scientific Directorate, Pediatric Hospital "Bambino Gesù", IRCCS, Rome, Italy

ARTICLE INFO

Article history:
Received 18 January 2010
Accepted 8 May 2010
Available online 26 June 2010

Keywords:
Adolescents
Children
Non-alcoholic fatty liver disease
Non-alcoholic steato-hepatitis
Portal inflammation

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].

* Corresponding author at: Liver Unit, Pediatric Hospital "Bambino Gesù", Via S. Onofrio 4, 00165 Rome, Italy. Tel.: +39 06 68 59 22 43; fax: +39 06 68 59 21 92.
E-mail addresses: nobili66@yahoo.it, usaip@medicina.unica.it (V. Nobili).
[1] These authors contributed equally to this work.

1590-8658/$36.00 © 2010 Editrice Gastroenterologica Italiana S.r.l. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.dld.2010.05.007

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	–	–	–	–

Table 2
Clinical and demographic data of the paediatric study population, stratified by degree of portal chronic inflammation.

	Portal chronic inflammation				
	None or mild ($n = 86$)		More than mild ($n = 58$)		Mann–Whitney test
	Median	IQR	Median	IQR	Exact p-value
Age (years)	12	4	12	4	0.440
Weight (kg)	64	27	63	28	0.674
Height (m)	1.50	0.20	1.50	0.20	0.399
BMI (kg/m^2)	26.3	6.5	26.5	5.7	0.906
BMI (SDS)	1.9	0.7	1.9	0.6	0.893
Waist (cm)	93	14	93	15	0.563
Percent body fat (%)	26	9	27	6	0.865
ALT (U/L)	68	48	67	35	0.706
AST (U/L)	43	24	44	24	0.799
GGT (U/L)	21	14	22	12	0.674
Glucose (mg/dL)	79	10	81	14	0.562
Insulin (μU/mL)	13	10	9	8	0.047
HOMA-IR	2.5	1.8	1.9	1.9	0.033
ISI	3.1	2.6	4.1	3.7	0.025
Triglycerides (mg/dL)	80	56	83	61	0.941
Cholesterol (mg/dL)	155	44	167	43	0.116
Systolic BP (mm Hg)	109	25	110	18	0.459
Systolic BP (SDS)	0.3	2.0	0.3	2.2	0.431
Diastolic BP (mm Hg)	69	11	70	11	0.311
Diastolic BP (SDS)	0.5	1.2	0.6	1.3	0.320
NAS	4	3	4	4	0.064

IQR = interquartile range; SDS = standard deviation score; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl-transferase, HOMA-IR = homeostasis-model assessment of insulin resistance; ISI = insulin sensitivity index; BP = blood pressure; NAS = NAFLD activity score.

Table 3
Distribution of histopathological outcomes in children with none, mild and more than mild portal chronic inflammation.

	Degree or stage of outcome							
	0		1		2		3	
	None or mild PCI, n (%)	More than mild PCI, n (%)	None or mild PCI, n (%)	More than mild PCI, n (%)	None or mild PCI, n (%)	More than mild PCI, n (%)	None or mild PCI, n (%)	More than mild PCI, n (%)
Steatosis	–	–	20 (51.28)	19 (48.72)	35 (60.34)	23 (39.66)	31 (65.96)	16 (34.04)
Lobular inflammation	8 (57.14)	6 (42.86)	59 (57.84)	43 (42.16)	16 (64.00)	9 (36.00)	3 (100.0)	0 (0.0)
Ballooning	41 (53.95)	35 (46.05)	18 (64.29)	10 (35.71)	27 (67.50)	13 (32.50)	–	–
Fibrosis	23 (56.10)	18 (43.90)	54 (62.07)	33 (37.93)	2 (40.00)	3 (60.00)	7 (63.64)	4 (36.36)

PCI = portal chronic inflammation.

The overall NAS score was also similar in the two groups. Fasting insulin and HOMA-IR were higher in children with none or mild PCI. Likewise, insulin sensitivity as detected by ISI, was lower in children with none or mild PCI. Thirty-two (22%) children had NASH. All children with NASH had overlap between type 1 and type 2 NASH [4].

To test for the presence of an association between more than mild PCI *vs.* no or mild PCI and the severity of the histopathological outcomes, we used an exact ordinal logistic continuation-ratio model. We found no association between PCI and the degree of steatosis, lobular inflammation, ballooning or the stage of fibrosis (Tables 3 and 4).

Table 4
Association of histopathological outcomes with portal chronic inflammation.

	Degree or stage of outcome (ordinal outcome with continuation-ratio logistic model)				
	0 OR (95% CI)	1 OR (95% CI)	2 OR (95% CI)	3 OR (95% CI)	4 OR (95% CI)
Steatosis	–	Ref. cat.	0.79 (0.53–1.18)	0.82 (0.55–1.21)	–
Lobular inflammation	Ref. cat.	0.94 (0.50–1.82)	0.81 (0.49–1.29)	0.61 (0.00–1.89)	–
Ballooning	Ref. cat.	0.77 (0.54–1.11)	0.80 (0.52–1.20)	–	–
Fibrosis	Ref. cat.	0.90 (0.61–1.35)	1.08 (0.59–1.95)	0.91 (0.41–1.87)	–

OR = odds ratio; 95% CI = exact 95% confidence interval; Ref. cat. = reference category for the ordinal outcome.

4. Discussion

The NASH CRN has recently reported an association between PCI and disease severity in children in the U.S.A. with NAFLD [6]. In the present study, however, we were not able to confirm the existence of such an association in an external series of Italian children with NAFLD.

It must be pointed out that our series of children differs substantially from the CRN one. Our children had a lower degree of obesity as compared to the CRN ones, as also reflected by lower values of fasting insulin and HOMA-IR (≈50%). Although the median values of ALT and AST were ≈30% lower in our children than in the CRN ones, we confirm their finding of a lack of association between ALT and the degree of PCI [6]. The NASH CRN study also found no association between BMI, markers of insulin resistance and PCI [6]. While we confirm the lack of association with BMI (which we report as SDS, not as absolute value as done by the NASH CRN), we found an association between PCI, fasting insulin, HOMA-IR and ISI. The association was inverse, i.e. the lower the degree of PCI, the higher the degree of insulin resistance. Although this finding was unexpected and may have occurred by chance owing to the large number of hypotheses being tested (Table 1), its size and the fact that children with no or mild inflammation had consistently higher values of glucose and insulin during OGTT (data not shown) suggests that, at least in our series, PCI is associated with less severe insulin resistance. However, considering the present lack of a biologically plausible explanation for this finding, further studies need to be performed on this topic.

Our findings cannot be easily attributed to any difference in anthropometry or body composition as our two groups of children were virtually identical in these measures (Table 1). We have however some tentative explanations for the discrepancies between our study and the NASH CRN one. First, because just two of our children had no PCI, we could not model the outcome as "none" *vs.* "mild" *vs.* "more than mild" as done by the NASH CRN study [6]. The dichotomization of the outcome in "none or mild" *vs.* "more than mild" that we were obliged to perform may be partly responsible for the lack of association between PCI and the histological outcomes. Second, ethnicity is emerging as a central risk factor for NASH severity and the NASH CRN series is characterized by a quite different ethnic mix as compared to our series of Caucasian children [6]. Moreover, genetic and environmental factors are likely to modulate the clinico-pathological associations of NAFLD in different populations [2].

In conclusion, we were not able to confirm the existence of a clinico-pathological association between PCI and disease severity in Italian children with NAFLD. Besides showing the need of performing further research on this important topic, our study suggests that at least some clinico-pathological correlates of paediatric NAFLD may be population-specific.

Source of support

"Bambino Gesù" Paediatric Hospital and Liver Research Center.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Dunn W, Schwimmer JB. The obesity epidemic and nonalcoholic fatty liver disease in children. Curr Gastroenterol Rep 2008;10:67–72.
[2] Alisi A, Manco M, Vania A, et al. Pediatric nonalcoholic fatty liver disease in 2009. J Pediatr 2009;155:469–74.
[3] Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, et al. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. Gut 2009;58:1538–44.
[4] Nobili V, Marcellini M, De Vito R, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. Hepatology 2006;44:458–65.
[5] Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005;42:641–9.
[6] Brunt EM, Kleiner DE, Wilson LA, et al. Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-clinicopathologic correlations from the nonalcoholic steatohepatitis clinical research network. Hepatology 2009;49:809–20.
[7] Hübscher SG. Histological assessment of non-alcoholic fatty liver disease. Histopathology 2006;49:450–65.
[8] Nobili V, Bedogni G, Alisi A, et al. A protective effect of breastfeeding on the progression of non-alcoholic fatty liver disease. Arch Dis Child 2009;94:801–5.
[9] Manco M, Bedogni G, Monti L, et al. Intima-media thickness and liver histology in obese children and adolescents with non-alcoholic fatty liver disease. Atherosclerosis 2010;209:463–8.
[10] Nobili V, Alisi A, Vania A, et al. The pediatric NAFLD fibrosis index: a predictor of liver fibrosis in children with non-alcoholic fatty liver disease. BMC Med 2009;7:21.
[11] Manco M, Bedogni G, Marcellini M, et al. Waist circumference correlates with liver fibrosis in children with non-alcoholic steatohepatitis. Gut 2008;57:1283–7.
[12] Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988.
[13] Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data 2000;314:1–27.
[14] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487–95.
[15] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–70.
[16] National High Blood Pressure Education Program Working Group on High Blood Pressure. The Fourth Report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114.
[17] Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.
[18] Ananth CV, Kleinbaum DG. Regression models for ordinal responses: a review of methods and applications. Int J Epidemiol 1997;26:1323–33.
[19] Hilbe J. Logistic regression models. Boca Raton: CRC Press; 2009.