Early Indexes of Damage Progression in the Pediatric Non Alcoholic Fatty Liver Disease: A Pilot Study

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Abstract

Background: Identification of factors influencing progression of Non-Alcoholic-Fatty-Liver disease (NAFLD) in children has an important clinical impact. This could allow clinicians to early identify a sub-group of children eligible for special monitoring programs, preventive therapeutic strategies and specific treatments.

Methods: To achieve these objectives, 60 consecutive children with a histopathology diagnosis of NAFLD or NASH were enrolled into this study. Subjects were divided into two groups: non- NASH group (n=29) and NASH group [NAS score >/=5 (n=31)]. Main demographic, clinical and laboratory’s features were analysed.

Results: Our data demonstrated that some patients ‘characteristics (ALT, ureic nitrogen/24h, BMI, Harrison Benedict kcal/day and VO2), independently on NAS score, were significantly associate with disease progression to fibrosis (p<0.05). The results showed that triglycerides represent an independent parameter of disease progression.

Conclusions: In our study, we identified a predictive model for NAFLD disease progression, based on inexpensive and easily available variables, potentially useful in routine clinical practice in the pediatric population.

Keywords: Liver disease, children, NAFLD, fibrosis, hepatic metabolism


Introduction

Over the last decades, the increasing prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD) has become the main cause of liver disease in the pediatric population worldwide. Epidemiological data report that between 3% and 11% of the pediatric population present NAFLD, with highest prevalence reached among overweight and obese children [1-4]. NAFLD constitutes a spectrum of related disorders characterized by corresponding histological features where steatosis might play a fundamental role in the progression to Liver Non-Alcoholic Steato-Hepatitis (NASH) and fibrosis in up to 41% of the cases according to published studies [2-3]. Although steatosis might be considered a benign course, however NAS has become a major childhood health-related issue as it can progress to liver cirrhosis. Approximately 8% of children undergoing liver biopsy for suspected NAFLD have already cirrhosis [4-7]. Currently, in pediatric age, liver biopsy remains the most accurate diagnostic method providing data on the degree of hepatic histological changes, for the diagnosis of NAFLD and related complications [8]. A histological system of scoring the features of NAFLD, called the NAFLD Activity Score (NAS), has been developed as a tool to measure changes in liver steatosis; the proposed NAS score clearly separates three lesions that comprise steatosis, lobular inflammation, and ballooning [9]. However, despite its sensibility, liver biopsy remains an invasive procedure though cannot be
extensively utilized for large scale population, due to obvious risks related to the procedure, especially in children. Based on this limit, the need for the early recognition of variables that predict the transition from NAFLD to NASH, in order to prevent further liver-related complications, constitutes an emerging problem. To date, serum aminotransferase levels associated to abdominal ultrasonography represent the recognized imperfect tools for diagnosing NASH. However, their accuracy and sensibility are still not able to early identify subclinical injuries underlying NAFLD-damage progression. Unfortunately, the mechanism of damage progression from a healthy status to NAFLD and NASH remains still unknown and somehow unpredictable. The most accepted pathogenetic hypothesis is the “multiparallel hits theory”, in which several factors (genetic, epigenetic, and environmental), interact leading to metabolic disarray that induces the onset of liver injury and its progression, independently on patient’s weight [10]. For this reason, whenever necessary, the time of liver biopsy is not definitely determined and this represents a central area of research for the near future. The aim of the present study was to evaluate the ability of early, specific and non-invasive laboratory parameters predicting the progression of liver damage, to potentially identify a sub-group of children deserving careful investigation.

Material and Methods

Patients and study design

The study was performed at the “Bambin Gesù Children’s Hospital” (Rome, Italy) during the period April 2013 to November 2013. Sixty consecutive children were enrolled into a prospectively maintained database and included in this study with a histopathology diagnosis of NAFLD or NASH. Subjects were divided into two groups according to their histopathological NASH status: non-NASH group (n=29) and NASH group [NAS score >/=5 (n=31)]. The study was performed in accordance with the principles of the Helsinki Declaration; all patients were assented and parents asked to give written consent to participate and have data collected. The study obtained the approval of the Local Ethical Committee of “Bambin Gesù Children’s Hospital”. Children with underlying organic causes of liver disease (hepatitis virus infection or other competing causes of hepatic steatosis and coeliac disease) and Type I diabetes, as well as on diet, were excluded from the study.

Demographic and clinical data

We obtained demographic data (including age at first visit and sex) and recorded the clinical variables, including waist circumference (WC) (cm), height (cm) and weight (kg). We calculated the body mass index (BMI) and its standard deviation score (z score) [11]. We defined obesity by a BMI ≥95th percentile adjusted for age and sex. Metabolic syndrome (MS) was defined as the presence of ≥3 of the following 5 criteria: abdominal obesity (defined by WC ≥90th percentile for age); hypertriglyceridemia as triglyceride (TG) >95th percentile for age, sex, and race [12, 13].

Laboratory and Ultrasound assessment

Laboratory assessment using standard lab-methods were performed and all the data were uniformly collected at the time of patient evaluation to avoid recall bias. Serum level of triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, γ-glutamyl-transpeptidase (γGT), uric acid, fasting glucose and insulin levels were measured after at least 12-hour fasting period at the first visit. All participants also underwent a standard oral glucose tolerance test, performed with 1.75 g of glucose/kg of body weight (up to 75 g). To estimate the individual’s metabolic rate, the following equations have been calculated: Respiratory Quotient (RQ), Resting Energy Expenditure (REE), Harrison Benedict equation, Homeostasis of Assessment of insulin resistance (HOMA) and the insulin sensitivity index. Moreover, blood samples for the measurement of adipokines and other cytokines were taken at enrolment, as part of the study protocol; leptin, adiponectin PBR4 and hyaluronic acid (HA) concentration were measured using a quantitative sandwich enzyme immunoassay (ELISA), according to the instructions of the manufacturer. Finally, Ultra Sound (US) was performed by a single radiologist, with a long-time experience in the field, to assess liver morphology and grading of steatosis.

Liver Biopsy

Liver biopsy was performed after an overnight fast, and specimens processed as previously detailed [14]. Biopsies were evaluated by a single blinded pathologist, with a long-time experience in the field. Steatosis, inflammation and hepatocyte ballooning were scored using the NAFLD Clinical Research Network criteria, as recommended. The patients received no treatment for NAFLD before liver biopsy was performed [15]. As recommended by current guidelines based on NAS score, biopsies were categorized by the NAFLD activity score, on the basis of overall injury pattern (steatosis, hepatocyte ballooning, inflammation) [16, 17].

Liver Disease Scores

Features of steatosis, lobular inflammation and hepatocyte ballooning were combined to obtain the NAFLD activity score (NAS) [17]. A simple, inexpensive and non-invasive test called PNFI has been also used, as simple algorithm for the prediction of liver fibrosis in children with NAFLD; as previously described, it combines standard biochemical values and age, to determine the significant presence of liver fibrosis.

Statistical analyses

All statistical analyses were performed using the statistic program STATISTICA 5.1 for Windows (Statsoft Inc.g). The median (interquartile range) or frequency (%) was used for descriptive statistics as appropriate. Independent continuous data were compared using the Mann-Whitney U test and the Kruskal-Wallis ANOVA as appropriate. Univariate correlation analysis was performed with the Spearman rank correlation test and multiple regression models were created with significant predictors to determine the independent contributions of the different liver fibrosis predictors. The variables that were significant at the univariate analysis were then included in
generalized regression linear models. All tests were two-sided and a p-value less than 0.05 was considered significant.

Results

Patient characteristics and multiple comparison data between sub-groups (Non-NASH vs. NASH groups)

A total of 60 children (62% boys) were included in the study; subjects were divided into two groups according to biopsy-proven liver-status: non-NASH group (n=29) and NASH group [NAS score >/=5 (n=31)]. The baseline anthropometric, clinical and laboratory data are summarized in Table 1. The mean age at the initial visit was 11.8±2.7 years; the non-NASH group consisted of a total of 29/60 (48.3%) children, while 31 (51.6%) were the NASH group.

21/60 (35%) children were obese and 22/60 (36.6%) overweight in the entire population (Table 1); obese patients were respectively 27% in the non-NASH group and 41% in the NASH group. BMI percentile between groups did not significantly differs (p=NS).

As expected, ALT level in non-NASH group was 28.5 U/L (mean value), significantly different from the NASH group (36.6 U/L) (p-value <0.05). Moreover, a significant difference has been evidenced also respectively for the following parameters: triglycerides, gGT and inflammatory markers such as hyaluronic acid levels and, inversely, leptin and adiponectin (p-value <0.05).

Histology

The mean NAS score was 4.7±1.4. Only 3/60 (5%) of patients had no inflammation activity at histology; on the contrary, the majority of children 57/60 (98%) showed inflammatory features and half of them [33(55%)] had mild severity of lobular inflammation, according to pathologist diagnosis. Ballooning was present in 85% of patients. Histological injuries were confirmed between groups also according to PNFI index and inflammatory serum markers (Hyaluronic Acid) (p-value<0.05).

Univariate analysis

Univariate analysis performed on the total population showed that lab characteristics (ALT, triglycerides, gGT and Hyaluronic acid) were significantly associate with disease progression to NASH (p<0.05).

Multivariate predictive model of disease progression to NASH

To better identify predictors of disease progression to NASH, we built a multivariate logistic regression model including all the variables significantly associated with disease progression to fibrosis at univariate analyses. Based on the results obtained from multiple comparison data between sub-groups non-NASH group (n=29) and NASH group [NAS score >/=5 (n=31)], the multivariate predictive model revealed that triglycerides remained still significantly associated with

### Table 1 (a&b): Patient characteristics and multiple comparison data between sub-groups (Non-NASH vs NASH groups).

<table>
<thead>
<tr>
<th>A</th>
<th>NON NASH group</th>
<th>NASH group</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>Mean</td>
<td>Median</td>
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<tr>
<td>Basal Glycaemia (mg/dl)</td>
<td>29</td>
<td>86,742</td>
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<tr>
<td>Mean glucose</td>
<td>29</td>
<td>94,138</td>
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<tr>
<td>Mean Ins</td>
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<td>Homa IR</td>
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<td>Harris-Benedict kcal/day</td>
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<td>npRQ</td>
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<td>REE (kcal/day)</td>
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<tr>
<td>RQ</td>
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<td>0,829</td>
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<td>Fibrosis index: fib4 index*</td>
<td>29</td>
<td>0,966</td>
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<table>
<thead>
<tr>
<th>B</th>
<th>NON NASH Group</th>
<th>NASH Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean</td>
<td>Median</td>
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<tr>
<td>Age</td>
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<td>Tryglycerides</td>
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<td>Leptin</td>
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<tr>
<td>Hyaluronim Acid</td>
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<td>1023,893</td>
</tr>
</tbody>
</table>

*P<0.05.
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Carraro A (2018) Early Indexes of Damage Progression in the Pediatric Non Alcoholic Fatty Liver Disease: A Pilot Study
Pediatr Res Child Health Volume 1(2): 2018

Discussion

NAFLD pathogenesis and progression remains an unsolved issue in both children and adults, presenting as a condition which might progress to NASH and cirrhosis or even cancer [4]. Currently, there is no specific biochemical or serological markers testing the progression of NAFLD, and liver biopsy remains the most accurate diagnostic method providing data on the degree of hepatic global changes. However, despite its sensibility, liver biopsy remains an invasive procedure which cannot be extensively utilized for large scale population. It is well reported that identifying predictive variables for liver disease progression represents a major objective of the worldwide pediatric community. Although overweight and obesity, especially in the setting of metabolic syndrome, might represent one of the most important factors contributing to the aggravation of NAFLD, however increasing evidence suggests that BMI is an imprecise measure of body-fat-related risk of liver diseases. Other features associated with overweight or obesity (visceral fat mass, adipose tissue inflammation, insulin resistance) might be the key pathogenetic aspects.

The most widely accepted paradigm proposed for NASH pathogenesis is the so-called “two-hit hypothesis”; based on this paradigm the primary abnormality is represented by the accumulation of triglyceride droplets within hepatocytes, as a consequence of insulin resistance [18]. The consequent hyperinsulinemia promotes de novo hepatic lipogenesis and an increased presence of free fatty acids in the liver [19, 20]. The resultant steatotic liver is then vulnerable to additional insults, the so-call “second hit” (oxidative stress, adipocytokines, and gut-derived bacterial endotoxins) [21, 22]. Based on this view, one of the major question in the pediatric population suffering from NAFLD remains who is the subgroup of patient deserving a strictly observation and a liver biopsy. Our study confirmed that BMI percentile between groups does not significantly differ (p=NS) and BMI cannot be considered an independent parameter to select patients with disease progression; also, ALT level in non-NASH group is significantly different from the NASH group even though, interestingly, the mean value in the NASH group (36.6 U/L) is less than the standard value considered for steatohepatitis. On the contrary, study groups showed a significant difference for triglycerides, gGT and inflammatory markers such as hyaluronic acid levels and, inversely, leptin and adiponectin (p-value <0.05); these data seem to be according with the two hits hypothesis, and should be considered the first sub-clinical alterations in NAFLD patient with disease progression. These data suggest that an anomalous lipid metabolism might determine the activation of an inflammatory cascade. If we consider the multivariate predictive model, it is clearly confirmed that triglycerides’ alterations are able to inversely predict disease progression to NASH with a god level of accuracy in the setting of NAFLD. These results are confirmed in especially for children with NAS score >5. This indirectly reflects that the decreased hepatic metabolism and the increased release from fat tissues are the most likely mechanisms of increased leptin levels in disease progression to NASH and fibrogenesis. On the other way adiponectin has been shown to be elevated in advanced liver disease independently of the etiology and we recently found to be independently associated with altered metabolism in advanced stages of NAFLD [23].

Our work showed no association of triglycerides and inflammatory markers with BMI or fat mass, suggesting that disease progression may affect also children apparently in good health. It seems that in NAFLD there is no increase production of adiponectin related to fat mass but the altered levels are linked to reduced hepatic metabolism. The activation of adipokines and the interplay with inflammatory factor may represent one of the most important trigger. In this scenario an altered lipid metabolism cooperates with several liver derived damaged-associate molecular patterns, including HA inflammatory cascade and inducing fibrogenesis. It is reasonable that children with altered sub-clinic metabolism, having a cytokine and inflammatory deregulations, may present a higher susceptibility to develop progression of liver injury. To our knowledge this is the first study to associate HA, adiponectin and leptin and, independently, triglycerides’ level to progression of liver diseases independently of BMI and the presence of a metabolic syndrome. This aspect may represent an important point to identify and select children under risk for disease progression without clear NASH status, determined by the standard diagnostic setting. However, our study has several limitations including the sample size, the absence of age-matched controls without NAFLD and, finally, the absence of a long-term patients’ follow-up. On this basis our findings need replication in larger and external samples to confirm these preliminary results and test the efficacy of early intervention in order to slow the progression of liver damage in children with NAFLD [24-29].

Conclusions

In conclusion, our results demonstrate that HA and adipokines are deregulated in liver disease progression in accordance to the degree of liver dysfunction in children with NAFLD disease and triglycerides represent an independent factor of disease progression to NASH. As such, triglycerides should be viewed as a substantial risk factor for the development of NASH and should be paid more attention to in future research. Targeted approaches should be developed to identify the subgroup children with altered triglycerides to establish a special monitoring program for NASH development.

Acknowledgements

The authors thank Professor Valerio Nobili for kindly providing datasets.

Conflicts of interest

The authors declared that there are no conflicts of interest.
References


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