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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Nonalcoholic fatty liver disease is associated with lower hepatitis B viral load and antiviral response in pediatric population

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Abstract

Background The interaction between nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis B infection (CBI) was unclear. We aimed to investigate the association between NAFLD and CBI and the effect of NAFLD on response to antiviral therapy in pediatric population.

Methods All children aged 0–18 years with liver biopsy-proven NAFLD, CBI, and co-existing NAFLD and CBI were consecutively collected. Children with co-existing CBI and NAFLD were considered as cases and *n:m* matched with simple NAFLD and simple CBI patients in the same cohort, respectively. In longitude study, the role of NAFLD in antiviral response was further analyzed in children with CBI who received antiviral treatment. Logistic or Cox regression models were used appropriately for analysis.

Results 765 subjects were finally enrolled with 62 co-existing patients, 560 CBI patients, and 143 NAFLD patients. Multivariate analysis showed that HBV DNA level was negatively associated with NAFLD in CBI children (OR 0.376, 95% CI 0.173–0.818). Conversely, the severity of steatosis and levels of serum lipid profile were found to be inversely associated with CBI in NAFLD subjects. Then, in longitude study, we found that HBsAg loss at 96 weeks of antiviral treatment was independently associated with NAFLD (aHR 3.245, 95% CI 1.288–8.176).

Conclusions An inverse association between CBI and NAFLD reciprocally existed in pediatric population. In longitude study, HBsAg loss was associated with NAFLD at week 96 of antiviral therapy.

Keywords Nonalcoholic fatty liver disease (NAFLD) · Chronic hepatitis B infection (CBI) · Lipid metabolism · Antiviral treatment · Pediatric population

Abbreviations

CBI	Chronic hepatitis B infection
NAFLD	Nonalcoholic fatty liver disease
BMI	Body mass index
DBil	Direct bilirubin
TBil	Total bilirubin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase

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ALP	Alkaline phosphatase
GGT	Glutamyl transferases
TBA	Total bile acid
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
Lp (a)	Lipoprotein (a)
PT	Prothrombin time
Peg IFN	Pegylated interferon
NA(s)	Nucleos(t)ide analogues

Introduction

Hepatitis B virus (HBV) infection is a major global health challenge, with estimation of 257 million infections worldwide. The risk of HBV chronicity is age dependent: up to 90% for infants and 50% for children < 6 years, compared with < 5% for adults [1]. Chronic HBV infection (CBI) in children characterized by higher level of viral replication and a greater risk of developing sequelae compared with those acquired during adulthood [2]. Although most CBI children were in immune-tolerant phase and the hepatic histology were shown normal or minor changes [3]. Despite the number of CBI children has declined benefiting from interruption of vertical transmission at birth and the implementation of vaccination program, there are still many children developing CBI owing to lack of access to immunization programs, which thwart the progress towards achieving the global hepatitis goals in 2030 that WHO launched [4].

Over the last decade, nonalcoholic fatty liver disease (NAFLD) has become another common chronic liver disease with a rapidly rising prevalence in Western and Asia–Pacific countries [5, 6]. The palpably increased incidence of NAFLD seems more remarkable in children and NAFLD has become the most common liver disease in children in the United States [7]. NAFLD in children presents unique histopathological characteristics which seem quite different from those in adult NAFLD, especially in younger age: more abundant or accentuated steatosis in zone 1 hepatocytes is manifested, and inflammation and fibrosis may be pronounced in portal tracts initially. Ballooning is not common [8]. These unique features may distinctly play roles in NAFLD progression or courses of other co-existing diseases [9–11].

Given that both NAFLD and CBI are the most prevalent liver diseases and the interaction between hepatic steatosis and HBV infection attracted the attentions of clinician. NAFLD is strongly associated with metabolic syndrome, which has been reported to be an independent risk factor for advanced fibrosis and cirrhosis in CBI patients [12]. CBI patients with high body mass index (BMI) is also unlikely to achieve fibrosis reversal after antiviral therapy and have high risk of liver-related mortalities than those without [13–15]. However, the effect of NAFLD per se on CBI is unclear. HBV viral load has been demonstrated to be inversely associated with incidence of NAFLD [16–19], while a number of biopsy-based studies failed to reveal any association between them [9, 20, 21]. On the other hand, the role of HBV infection in NAFLD also remains controversial. Large cohort studies demonstrated that the prevalence of NAFLD is lower in HBV patients than in uninfected patients [22]. While in status of HBV infection, hepatic steatosis is only associated with metabolic but not viral factors, suggesting no effect of HBV virology on steatosis. Investigations of the association between steatosis and HBV infection have been mainly limited to adult population. With the increasing rate of NAFLD in children and the remaining considerable size of CBI pediatric population, we aimed to investigate the interplay between steatosis and HBV infection in children by comparing the viral factors and metabolic factors between CBI children with or without NAFLD, as well as NAFLD children with or without CBI. We also aimed to clarify the role of NAFLD on the outcome of antiviral treatment in CBI children.

Methods

Patients

All children aged 0–18 years with liver biopsy-proven NAFLD were retrospectively and consecutively analyzed from January 2010 to March 2018 at the 5th Medical Center, Chinese PLA General Hospital. We also consecutively recruited naïve CBI children with liver biopsies during the same time in the same hospital. Exclusion criteria included children with: (1) malignant hepatic tumor; (2) acute hepatitis B; (3) anti-HBV treatment; (4) suspected or confirmed hepatolenticular degeneration; (5) co-infection with other viruses; and (6) other hepatic disease or severe systemic disease. Exclusion criteria for NAFLD were genetic/metabolic disorders, infections except HBV, use of steatogenic medications, ethanol consumption, or malnutrition.

Patients with co-existing CBI and NAFLD were considered as cases and *n*:*m* matched with simple NAFLD and simple CBI patients by age and sex in the same cohort, respectively.

Study design

Demographic, clinical, and routine biochemistry data at inclusion were recorded. Two comparisons were performed. We first compared all the HBV parameters

between CBI children with or without NAFLD to determine the association of NAFLD and severity of CBI. The second comparison was performed between NAFLD children with or without CBI to determine the association of CBI and degree of hepatic steatosis, incidence of metabolic syndromes, and serum lipid profiles.

CBI children with or without NAFLD who have disciplinary antiviral treatment course from January 2010 to January 2016 in this cohort were followed up for the HBV DNA loss, HBeAg loss, HBsAg loss, and HBsAg seroconversion. Only patients who have follow-up data at least every 24 weeks from the beginning to the end of 96 weeks' treatment were eligible for this study. Treatment strategy was recorded and classified as Peg IFN monotherapy and Peg IFN and NA(s) combination therapy. Children with NA(s) monotherapy were excluded owing to little available data. CBI children with and without NAFLD were *n*:*m* matched by age, sex, HBV profiles, status of necroinflammation, and fibrosis at baseline (Supplementary Table 1).

Definitions

Chronic HBV infection (CBI) is defined by positive serum hepatitis B surface antigen (HBsAg) for 6 months or more in accordance with American Association for the Study of Liver Diseases (AASLD) 2018 Hepatitis B Guidance [23]. Diagnosis criteria of NAFLD were based on NAFLD Practice Guidance from the AASLD and all enrolled subjects were proven by liver biopsy [8]. The diagnosis of metabolic syndrome was in accordance with IDF consensus report [24]. HBsAg loss is defined by undetectable HBsAg by commercially available assays (< 1 COI). HBeAg loss is defined by HBeAg undetectable (< 1 COI). HBV DNA less than 12 copy/mL was considered undetectable level. HBsAg seroconversion is defined by HBsAg loss and anti-HBsAg appear (> 1 COI). The upper limit values of normal ALT and AST were set to be 35 and 30 U/L, respectively.

Histological evaluation

Simple CBI patients were histologically assessed by METAVIR scoring system; simple NAFLD patients who underwent liver biopsy were evaluated by NASH CRN system; individuals co-existed with CBI and NAFLD were assessed by METAVIR scoring system for necroinflammation and fibrosis and NASH CRN system for steatosis scoring. All participants or their parents/ guardians have written informed consent.

Statistical analysis

Statistical analysis was performed using SPSS software for Windows version 21.0 (SPSS Inc., Chicago, IL, USA). Demographic data and baseline characteristics were presented as median and interquartile range. Continuous variables compared each group were used nonparametric Mann–Whitney *U* test, and categorical variables used Chi-square test or Fisher's exact test, as appropriate. Multivariate analyses were performed using *n*:*m*-matched conditional logistic regression, in which factors with $P < 0.05$ in the univariate model and the factors what we focus on were entered into a forward stepwise selection multivariate model. Variance inflation factor testing was used for the detection of multicollinearity and the value was no more than 10. Cox regression was built to explore the independent factors for HBsAg loss, HBeAg loss, and HBV DNA undetectable during the 96 weeks' antiviral treatment. A two-tailed p value < 0.05 was considered statistically significant.

Ethical statement

This investigation was performed according to the guidelines of the Declaration of Helsinki. This study has been approved by the local Ethics Committee.

Results

Patient characteristics

A total of 1558 children with CBI and/or steatosis were evaluated during the study period, of which 1156 subjects were eligible for the study according to the excluding criteria. Among these, 62 subjects had co-existing CBI and NAFLD. These sub-cohort patients were matched *n*:*m* with simple NAFLD and simple CBI patients in the same cohort, leading to 560 CBI patients and 143 NAFLD patients included (Fig. 1). Clinical characteristics of all enrolled children at baseline are reported in Table1. The median age was 6, 10, and 11 years in co-existing, NAFLD, and CBI groups, respectively, with a male predominance in each group.

Associated factors of NAFLD in CBI patients

In patients with CBI, AST level was higher and GGT level was lower in NAFLD co-existing patients than in those without NAFLD. The median BMI was 19.33 in co-existing group and 17.01 in simple CBI group ($P = 0.001$). Serum triglyceride, but not other metabolic parameters, was higher in CBI patients with NAFLD than those without. There existed no difference between the two groups in terms of HBsAg level, the rate of HBeAg positivity, HBV DNA copy number, and HBV genotype distribution. While

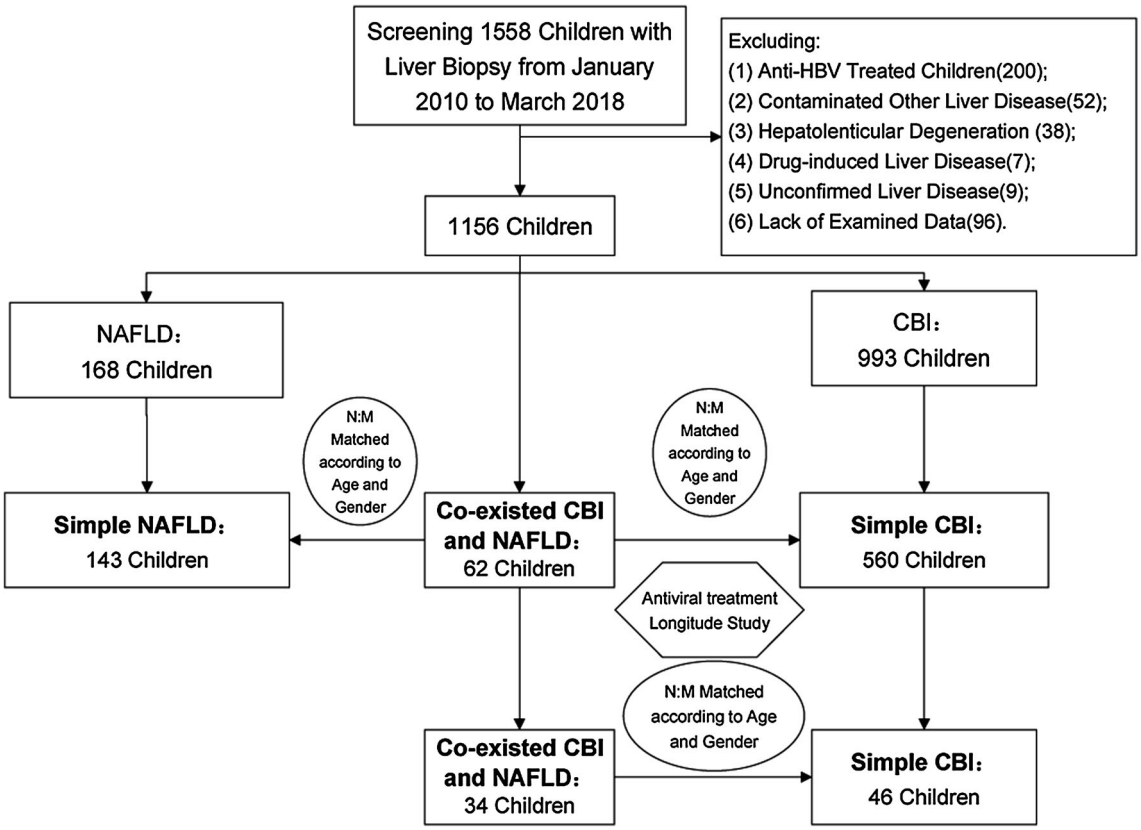

Fig. 1 Flow chart of the pediatric subjects enrolled in the study

the level of HBeAg was significantly lower in CBI children with NAFLD compared with simple CBI children ($P = 0.003$). In addition, the grade of inflammation and fibrosis was similar between the two groups. (Table 1) Then, HBV DNA copy was divided into high-level copy group ($> 10^5$ copy/mL) and low-level copy group ($\leq 10^5$ copy/mL) according to HBV-replicated activity. Interestingly, in multivariate analysis, low level of HBV DNA copy number was identified an independent factor associated with higher risk of NAFLD in CBI children [odd ratio (OR) 0.376, 95% confidence interval (CI) 0.173–0.818], adjusted for BMI (*Z*-score), albumin, prealbumin, globulin, AST, GGT, creatinine, urea acid, fibronectin, inflammation, fibrosis, and metabolic factors, suggesting a reverse associated between NAFLD and HBV viral load. Other independently associated factors of NAFLD in CBI children were BMI (*Z*-score) (OR 1.783, 95% CI 1.429–2.224), globulin (OR 1.075, 95% CI 1.003–1.153), prealbumin (OR 1.006, 95% CI 1.001–1.012), and creatinine (OR 0.948, 95% CI 0.920–0.976) (Table 2).

Associated factors of CBI in NAFLD patients

We further determined the associated factors of CBI in NAFLD patients. In NAFLD pediatric subjects, univariate analysis showed that NAFLD with CBI group has lower level of median ALT, GGT, platelet, and prealbumin compared to NAFLD without CBI group. In analysis of metabolic parameters, we observed BMI, level of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), and MS incidence were all lower in NAFLD patients with CBI than that in NAFLD without CBI patients (Table 1). Consistently, NAFLD children with CBI were less likely to have severe steatosis (8.1% vs.72.0%, $P = 0.001$). However, co-existing CBI was associated with severe inflammation than NAFLD children ($P = 0.046$). Nevertheless, multivariate analysis indicated that co-existing CBI remained associated with lower frequency of severe steatosis after adjusting for BMI (*Z*-score), blood platelet, albumin, prealbumin, TBA, GGT, TBil, creatinine, cholinesterase, uric acid, TC, LDL-C, ApoB, PT, fibronectin, inflammation, and fibrosis, suggesting an inverse association between steatosis and CBI in children with NAFLD (compared to mild, moderate OR 0.367, 95% CI 0.169–0.800; severe OR 0.089, 95% CI 0.032–0.244). Furthermore, prealbumin was also inversely associated with CBI (OR 0.993, 95% CI 0.988–0.999) (Table 3).

Table 1 Characteristics of pediatric population

Variable	CBI with NAFLD ($n = 62$) M (quartile)	CBI ($n = 560$) M (quartile)	p value*	NAFLD ($n = 143$) M (quartile)	p value#
Age (years)	11.0 (6.8–15.3)	6.0 (3.0–13.0)	< 0.001	10.0 (12.0–14)	0.638
Gender (M/F)	54/8	494/66	0.836	132/11	0.294
BMI (kg/m^2)	19.33 (16.67–24.51)	17.01 (15.38–19.13)	0.001	24.78 (22.15–27.55)	< 0.001
BMI (*Z*-score)	0.01 (− 0.56,1.12)	− 0.48 (− 0.83, − 0.03)	< 0.001	1.17 (0.61, 1.77)	< 0.001
Hemoglobin (g/L)	131.5 (125.0–145.3)	130.0 (123.0–140.0)	0.104	137.0 (132.0–144.0)	0.071
Platelet (10^9/L)	248 (208–286)	248 (210–301)	0.823	287.0 (249.0–335.0)	< 0.001
Albumin (g/L)	42.5 (40.0–47.0)	42.0 (39.0–45.0)	0.044	45.0 (43.0–47.0)	0.016
Globulin (g/L)	26.5 (24.0–29.0)	24.0 (22.0–27.0)	< 0.001	27.0 (24.0–29.0)	0.209
Prealbumin (mg/L)	151 (137–185.3)	141 (120–169)	0.02	232.0 (207.0–268.0)	< 0.001
DBil (umol/L)	2.95 (2.50–4.23)	2.65 (1.80–4.30)	0.067	3.3 (2.4–4.7)	0.466
TBil (umol/L)	7.7 (6.9–1.4)	7.6 (5.6–11.0)	0.148	8.8 (6.3–12.4)	0.712
ALT (U/L)	87 (51–124)	103 (58–203)	0.051	143 (89–248)	< 0.001
AST (U/L)	68 (46,87)	82 (54–145)	0.010	79 (50–129)	0.098
ALP (U/L)	263 (200–330)	264 (212–320)	0.830	278 (196–356)	0.533
GGT (U/L)	25 (21–45)	21 (14–40)	0.017	60 (42–97)	< 0.001
TBA (umol/L)	7 (5–13)	7 (4–13)	0.311	6 (3–9)	0.002
Creatinine (umol/L)	49 (44–61)	46 (39–60)	0.041	55 (47–63)	0.041
Cholinesterase (U/L)	7359 (6710–8925)	7323 (6344–8598)	0.272	9888 (8633–10,825)	< 0.001
Amylase (U/L)	53 (40–62)	54 (40–67)	0.363	47.5 (61.0–36.0)	0.325
Urea (mmol/L)	3.9 (3.5–4.6)	3.9 (3.3–4.7)	0.629	3.8 (3.2–4.4)	0.160
Urid acid (umol/L)	291 (271–369)	268 (221–327)	0.001	382 (307–444)	< 0.001
TC (mmol/L)	3.7 (3.4–4.3)	3.6(3.2–4.1)	0.116	4.36 (3.88–5.01)	< 0.001
TG (mmol/L)	0.90 (0.78–1.28)	0.88 (0.67–1.12)	0.047	1.47 (1.15–2.04)	< 0.001
HDL-C (mmol/L)	1.20 (1.03–1.29)	1.20 (1.00–1.37)	0.374	1.10 (0.97–1.31)	0.183
LDL-C (mmol/L)	2.31 (2.06–2.84)	2.31 (1.93–2.67)	0.379	2.96 (2.54–3.47)	< 0.001
Apo A1 (mmol/L)	1.28 (1.15–1.38)	1.28 (1.16–1.40)	0.763	1.22 (1.11–1.37)	0.246
Apo B (mmol/L)	0.61 (0.53–0.72)	0.61 (0.51–0.73)	0.757	0.81 (0.67–0.99)	< 0.001
Lp (a) (mmol/L)	62 (29–76)	62 (34–122)	0.088	46 (28–84)	0.372
PT(s)	11.4 (10.9–12.1)	11.4 (10.8–12.0)	0.440	10.9 (10.4–11.4)	< 0.001
Fibrinogen (g/L)	2.74 (2.22–3.28)	2.36 (1.92–2.83)	< 0.001	3.10 (2.57–3.54)	0.020
HBsAg (COI)	2162 (1108–5256)	2084 (970–4268)	0.211	–	–
HBeAg (COI)	410 (6.4–1026)	1011 (77–1398)	0.003	–	–
HBeAg (−) (*n*, %)	11 (21.57%)	66 (13.31%)	0.219	–	–
HBV DNA (copy/mL)	8.79 (0.84–23.53) × 10^7	7.46 (1.12–28.38) × 10^7	0.488	–	–
HBV genotype			0.451	–	–
B	12	101		–	–
C	34	383		–	
Grade of Necro.			0.840		0.046
A0–1	28 (45.2%)	198 (35.4%)		87 (60.8%)	
A2–3	34 (54.8%)	362 (64.6%)		56 (39.2%)	
Stage of fibrosis			0.691		1.000
F0–1	30 (48.4%)	286 (51.1%)		69 (48.3%)	
F2–4	32 (51.6%)	274 (48.9%)		74 (51.7%)	
Hepatic steatosis					0.000
Mild	46 (74.2%)	–		7 (4.9%)	
Moderate	11 (17.7%)	–		33 (23.1%)	
Severe	5 (8.1%)	–		103 (72.0%)	

Table 1 continued

Variable	CBI with NAFLD ($n = 62$) M (quartile)	CBI ($n = 560$) M (quartile)	p value*	NAFLD ($n = 143$) M (quartile)	p value#
Metabolic syndrome	1 (1.6%)	0		27 (18.9%)	0.001

CBI chronic hepatitis B infection, *NAFLD* nonalcoholic fatty liver disease, *Nero.* necroinflammation, *BMI* body mass index, *DBil* direct bilirubin, *TBil* total bilirubin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *GGT* glutamyl transferases, *TBA* total bile acid, *TC* total Cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *ApoA1* apolipoprotein A1, *ApoB* apolipoprotein B, *Lp (a)* lipoprotein (a), *PT* prothrombin time, *Grade of Necro.* Grade of Necroinflammation

*Means the p value between CBI with NAFLD group and CBI group,

#Means the p value between CBI with NAFLD group and NAFLD group

Table 2 Associated factors with NAFLD in 622 CBI pediatric individuals (multivariate analysis)

Variable	OR*	95% CI	p value
BMI (*Z*-score)	1.783	1.429–2.224	0.000
Globulin	1.075	1.003–1.153	0.042
Prealbumin	1.006	1.001–1.012	0.014
Creatinine	0.948	0.920–0.976	0.000
HBV DNA copy ($> 1.0*10^5$ copy/mL)	0.376	0.173–0.818	0.014

*Adjusted for BMI (*Z*-score), albumin, prealbumin, globulin, AST, GGT, creatinine, urea acid, fibronectin, inflammation, fibrosis, and lipid metabolic-related factors

Table 3 Associated factors with CBI in 205 NAFLD pediatric subjects (multivariate analysis)

Variable	OR*	95% CI	p value
Prealbumin	0.993	0.998–0.999	0.019
Hepatic steatosis			
Mild	–	–	0.000
Moderate	0.367	0.169–0.800	0.012
Severe	0.089	0.032–0.244	0.000

*Adjusted for BMI (Z-score), blood platelet, albumin, prealbumin, TBA, GGT, TBil, creatinine, cholinesterase, urid acid, TC, LDL-C, ApoB, PT, fibronectin, inflammation, and fibrosis

The relationship of CBI and serum lipid metabolism

NAFLD was mostly accompanied by disorder of lipid metabolism. We further investigated the burden of serum lipid profile in NAFLD children with or without CBI. We have previously shown that CBI was significantly associated with lower serum lipids and mild steatosis in children with NAFLD. Since hepatic steatosis is well known to strongly associate with serum lipids, we further stratified NAFLD patients according to the severity of hepatic steatosis and assess lipid profiles between NAFLD patients with or without CBI at the same hepatic steatosis status. Interestingly, CBI seemed still inversely associated with TG, TC, LDL-C, and Apo B, regardless of hepatic steatosis level in NAFLD patients and this association was stronger in patients at mild steatosis group vs. moderate and severe steatosis group (Fig. 2).

The data indicated that HBV may play some protective roles in metabolic disorder in children with NAFLD. Then, we followed up the serum level of lipid factors in 80 CBI patients who received 96 week antiviral treatment. However, we failed to establish the association between lipid factors and HBV viral variety or clearance, as demonstrated by the trend for lower lipids profiles with HBV suppression (Supplementary Fig. 1).

Factors associated with the effect of antiviral treatment

We next studied the role of NAFLD in antiviral treatment in children with CBI. 34 CBI children with NAFLD received antiviral treatment, which were *n*:*m* matched with 46 CBI children without NAFLD. In terms of treatment regimens, 23 received Peg IFN monotherapy and 57 received Peg IFN and NA(s) combination therapy.

At week 96 of antiviral treatment, there was no significant difference between CBI patients with or without NAFLD with regard to the rate of HBV DNA undetectable, HBeAg loss, HBsAg loss, and normalization of ALT and AST (Supplementary Table 2).

We then determined the independent predictors for favorable outcome of antiviral treatment. In multivariate analysis, after adjusting for age, gender, BMI (*Z*-score), UA, ALT, AST, medication, basal HBV profiles, inflammation, and fibrosis, NAFLD was unexpectedly found to be an independent predictor for HBsAg loss (aHR 3.245, 95%

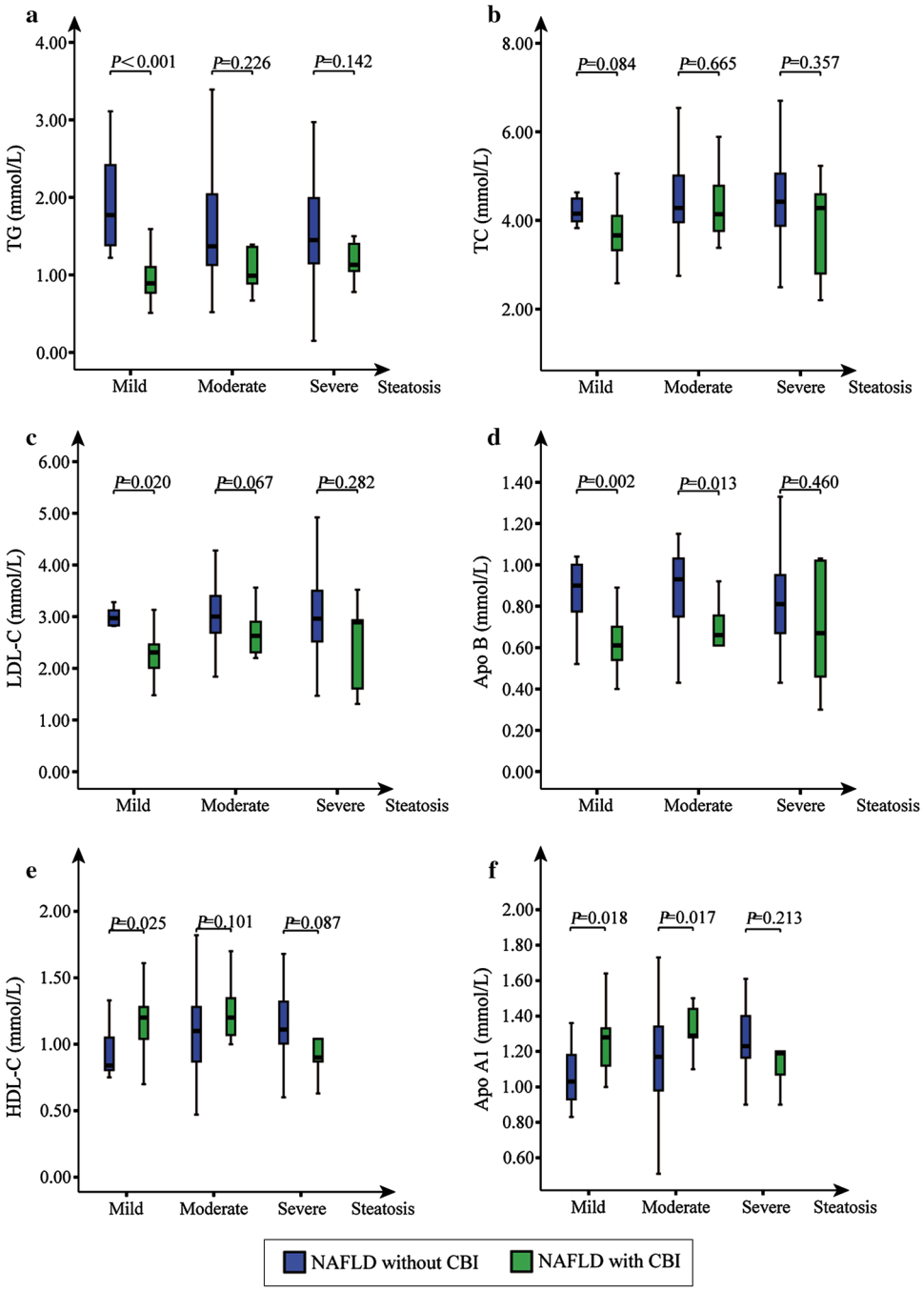

Fig. 2 Comparison of lipid metabolism indexes between NAFLD children with or without CBI. Children with NAFLD were stratified according to the severity of steatosis and the levels of TG (**a**), TC (**b**), LDL-C (**c**), ApoB (**d**), HDL-C (**e**), and ApoA1 (**f**) were shown between NAFLD patients with (green) or without (blue) CBI

CI 1.288–8.176) (Table 4). Consistently, the rate of HBsAg loss was higher in CBI patients with NAFLD than that without NAFLD at each follow-up timepoint. In addition, age (aHR 0.762, 95% CI 0.657–0.885), female (aHR 3.338, 95% CI 1.228–7.696), UA (aHR 0.991, 95% CI 0.982–1.000) and Peg IFN and NA(s) combination therapy (aHR 0.341, 95% CI 0.144–0.805) were also independently associated with HBsAg loss. In addition, the independent predictors for HBeAg loss were age (aHR 0.843, 95% CI 0.782–0.908) and AST (aHR 2.421, 95% CI 1.125–5.208). (Table 4).

Discussion

This study, with its unique pediatric cohort, biopsy-proven hepatic evaluation, long-term follow-up of antiviral treatment, explored the association between CBI and NAFLD in pediatric case–control population. The results demonstrate that HBV DNA level is inversely associated with prevalence of NAFLD in children with CBI. In parallel, we also found a negative association between steatosis severity and CBI in children with NAFLD. Of great interest, in longitude study, we found co-existing with NAFLD is

Table 4 Associated factors with antiviral treatment in 80 CBI pediatric subjects (multivariate analysis)

Variable	Factors	aHR*	95% CI	*p* value
HBV DNA undetectable				
	None	–	–	–
HBeAg loss				
	AST > 2*ULN	2.421	1.125–5.208	0.024
	Age	0.843	0.782–0.908	0.000
HBsAg loss				
	Female	3.338	1.448–7.696	0.005
	NAFLD	3.245	1.288–8.176	0.013
	UA	0.991	0.982–1.000	0.042
	Age	0.782	0.657–0.885	0.000
	Combination therapy	0.341	0.144–0.805	0.014
HBsAg seroconversion				
	UA	0.985	0.975–0.996	0.007

ULN upper limits of normal, *UA* uric acid

*Adjusted for age, gender, BMI (Z-score), UA, ALT, AST, medication, HBV profiles, inflammation, and fibrosis

associated with HBsAg loss at week 96 of antiviral therapy in CBI children.

The previous study analyzed the relationship between HBV infection and NAFLD mostly in adult population, with controversial results. Comparable prevalence of fatty liver was reported between HBsAg carriers and health controls [15, 25]. While a higher prevalence of fatty liver in CBI patients compared with healthy population was also reported. However, contrasting evidence has been brought forward by different investigators, demonstrating that the prevalence of hepatic steatosis diagnosed by ultrasound was lower in HBsAg carriers than in health controls [18, 22, 26]. Interestingly, one of the studies further revealed a strong inverse association between HBV DNA level and hepatic steatosis in HBV-infected adult patients, which is consistent with our results in pediatric CBI population. Similarly, another large case–control study reported that steatosis diagnosed by controlled attenuation parameter (CAP) assessment was an independent factor inversely associated with serum HBV DNA copy in HBV-infected patients (12). The explanation underlining the intricacies remains obscure. There are several ways of interpreting the inverse association: (a) a healthier lifestyle in HBV patients than general population could be taken into account; (b) a mechanistically cause–effect relationship may exist. Some studies demonstrated that fat accumulation in liver obviously reduces HBV DNA in animal models, suggesting a protective role of steatosis in HBV replication [19, 27]; and (c) an opposite cause–effect relationship may exist that HBV probably influences lipid metabolism and steatosis and is associated with lower risk of developing NAFLD [19]. This is in line with evidences from our study and others, as demonstrated by an inverse association between HBV infection and lipid profile including cholesterol, triglycerides, HDL-C, and LDL-C, consequently favoring the development of fatty liver. More molecular pathway studies with animal models are warranted to clarify whether and how the causal relationship exists.

HBV infection has been associated with alterations in lipid metabolism, demonstrated by the inverse association of HBV infection and lipid profiles [22, 2829]. Patients with CBI infection have been reported to harbor lower TG and HDL-C compared with health controls [28]. In a study of 7,695 individuals enrolled from health examination, HBsAg was inversely associated with levels of cholesterol, TG, and LDL-C [29]. Likewise, a prospective large cohort study from Korea reported that TG was lower and HDL-C was higher in HBsAg (+) carriers than HBsAg (−) individuals [22]. Similarly, in the context of NAFLD patients in our study, there is an inverse association between HBV infection and the lipid profiles even after adjusting for steatosis stages. These results suggested that the degree of serum lipid abnormality is conceivably related to HBV infection regardless of the steatosis severity. Some studies have proposed that lipids were largely consumed by HBV to provide energy for viruses envelop assembling, which probably explained our finding [16, 26]. However, in a time-dependent analysis in CBI children with NAFLD, we did not observe exacerbated lipid profiles with the updated HBV DNA copy number during antiviral treatment course, precluding a direct effect of HBV on lipid metabolism. According to the previous study, adiponectin, which is a protein produced by adipose tissue and inversely related to several metabolic disorders via possessing anti-inflammatory effects, maybe involved in the mechanistic interactions between the HBV molecules and the less severity of dyslipidemia, because the previous clinical reports suggested that presence of chronic HBV infection is positively associated with serum adiponectin levels and this finding was also congruent with study of experimental animal model that HBV replication can upregulate adiponectin [30–32]. However, the mechanism of inter-relationship between HBV component and lipid profiles remained to be better established.

Another important clinical concern is whether the hepatic steatosis influences the response to antiviral therapy. HBsAg loss or seroconversion was generally considered as the clinical therapeutic endpoint of antiviral therapy. Recent studies found that hepatic steatosis and related metabolic factors may play some roles in HBsAg loss. Moderate–severe steatosis has been found to contribute to HBsAg loss even without antiviral treatment [12]. A 3084

community-based study showed that extreme obesity (BMI $\geq$ 30 kg/m^2) is a positive predictor for HBsAg loss with a Cox multivariate-adjusted rate ratio of 1.51 (95% CI 1.09–2.08) [33]. Our finding compares well with the previous reports of implying a favor role of steatosis on HBsAg loss. Although the proportion of HBsAg loss is comparable between CBI children with or without NAFLD, NAFLD was found to be an independent factor associated with HBsAg loss after adjusting for age, gender, BMI (Z-score), UA, ALT, AST, medication, and HBV profiles at baseline. NAFLD seems to play a complex role in antiviral host response in patients with CBI. HBsAg-specific cytotoxic T lymphocytes (CTL), especially HBV-specific CD8 (+) T lymphocytes, are numerically and functionally insufficient to achieve complete viral clearance in patients with CBI [34–36], while the T lymphocytes were shown to be proliferated and activated in liver with altered lipid metabolism, which might promote HBV clearance in CBI-co-existed NAFLD individuals [37, 38]. The deficiency of innate immunity manifested by functionally impaired NK cell and arrested cytokines, such as IFN-γ and TNF-α, was also found in patients with CBI and involved in persisting HBV replication [39, 40], while fat accumulation is capable of promoting innate immune by activating damage-associated molecular patterns (DAMPS), pathogen-associated molecular patterns (PAMPs), and its receptors and subsequently activating both Kupffer cells (KC) and hepatic stellate cells (HSC) to produce inflammatory cytokines and chemokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) [38]. Thus, the activation of innate and adaptive immune induced by NAFLD may facilitate HBsAg clearance. In addition, in CBI individuals with NAFLD, hepatic steatosis is able to induce inflammation and apoptosis to clear HBV-infected hepatocytes [41, 42], which may facilitate HBsAg clearance. Moreover, Fas, one of the quintessential member of death receptor family, is documented to be up-regulated in patients with NASH [43]. Fas could increase cellular apoptotic-susceptibility, thus resulting in potent viral clearance in HBV-infected hepatocytes with steatosis [44, 45]. Consistently, according to our first part cross-sectional study, co-existing NAFLD is inversely associated with HBV DNA copy level in patients with CBI, presumably reflecting the suppressive effect of NAFLD on HBV viral replication. Therefore, CBI patients with NAFLD are likely to have favorable outcomes of antiviral treatment. However, the result should be interpreted with caution, as the number of patients is small in each subgroup and treatment regimens are heterogeneous in our longitude study cohort. Large-scale cohort studies are required to address the role of steatosis on antiviral response and additional work is needed to corroborate the mechanism and to provide deeper insight into whether metabolism can be a target for improving anti-HBV treatment.

There are some limitations in the current study. First, this is a retrospective study; thus, the data from the cross-sectional analysis between NAFLD and HBV limited establishment of the cause–effect association. Second, our finding is based on hospitalized pediatric population with liver biopsy, which is likely to produce biases in selection of population with severe diseases. Taken together, a prospective pediatric cohort with less influence of individuals' biases is warranted to resolve these uncertainties. Nevertheless, we believe that the valuable data from pediatric population in the current study provided perspectives for understanding the nature of the association between NAFLD and HBV infection.

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