Analysis of islet beta cell functions and their correlations with liver dysfunction in patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)

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Abstract

Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) primarily manifests in neonates or infants with hepatomegaly, liver dysfunction, and hypoglycemia. This study investigated the functions of islet beta cells and their correlations with liver dysfunction in NICCD patients.

We retrospectively analyzed clinical data on liver function and islet beta cell functions for 36 patients diagnosed with NICCD and 50 subjects as the control group. The NICCD group had significantly higher total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), aspartate amino transferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and alpha-fetoprotein (AFP) levels and albumin/globulin ratio (A/G) ($P < .05$), and lower ALB and GLB levels than the control group ($P < .05$). The differences in fasting blood glucose (FBG), fasting insulin, C-peptide (C-P), the homeostasis model of assessment for the insulin resistance index (HOMA-IR), fasting beta cell function (FBCI), and the HOMA beta cell function index (HBCI) between the NICCD and control groups were not significant ($P > .05$). A linear correlation was found between FBG and fasting insulin ($P < .001$) and between FBG and C-P in the NICCD patients ($P = .001$). Fasting insulin ($P = .023$), HOMA-IR ($P = .023$), FBCI ($P = .049$), and HBCI ($P = .048$) were positively correlated with increases in the ALT level. There was no difference in islet beta cell functions between the NICCD and control groups. The liver dysfunction may be correlated with islet beta cell functions in NICCD patients.

Abbreviations: A/G = albumin/globulin, AFP = alpha-fetoprotein, ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate amino transferase, C-P = C-peptide, CTLN2 = adult-onset citrullinaemia type II, DBIL = direct bilirubin, FBCI = fasting beta cell function, FBG = fasting blood glucose, FTTDCD = failure to thrive and dyslipidemia caused by citrin deficiency, GGT = gamma-glutamyl transpeptidase, GLB = globulin, HAS = human serum albumin, HBCI = the HOMA beta cell function index, HOMA-IR = the homeostasis model of assessment for the insulin resistance index, IBIL = indirect bilirubin, IR = insulin resistance, MCT = medium-chain triacylglycerol, NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency, TBIL = total bilirubin.

Keywords: islet beta cells, liver dysfunction, neonatal intrahepatic cholestasis caused by citrin deficiency

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1. Introduction

SLC25A13 gene mutations can lead to citrin deficiency (CD)[1], which is one of the main causes of infant cholestatic jaundice in China.[2] Three CD age-dependent phenotypes have been described[3–5]: neonatal intrahepatic cholestasis caused by CD (NICCD), adult-onset citrullinaemia type II (CTLN2), and failure to thrive and dyslipidemia caused by CD (FTTDCD).

NICCD primarily manifests in neonates or infants with the clinical conditions hepatomegaly, liver dysfunction, and hypoglycemia,[6,7] most of which have a good prognosis if they are diagnosed in a timely manner. CTLN2 presentation as an acute hepatic and neurological disorder in adolescents or adults (11–79 years of age) usually signifies an ominous prognosis.[6,8,9] FTTDCD occurs at the post-NICCD but pre-CTLN2 stage, and presents with failure to thrive, hyperlipidaemia, hepatoma, and pancreatitis.[3,10,11] After the NICCD period, some individuals may progress to FTTDCD, and some may develop CTLN2 within 1 decade or more.[12,13] The first case of NICCD in mainland China was reported in 2006.[14] Currently, NICCD is recognized as a common cause of infant cholestatic jaundice.[5,15] The accumulation of galactose, galactonic acid, galactitol, and other metabolites in the body cause an increase in hepatocyte osmotic pressure and hepatocyte enlargement and a decline in hepatocyte function. NICCD patients commonly present with liver enlargement, jaundice, hepatitis, and other abnormal liver

functions. The liver is an important organ for glucose metabolism; therefore, liver dysfunction due to NICCD may lead to a glucose metabolism disorder. Approximately 50% to 80% of insulin is metabolized in the liver, and hypoglycemia is common in NICCD patients.[16] However, whether liver dysfunction is due to a disturbance in insulin metabolism that results in hyperinsulinemia and then hypoglycemia is not fully understood. To the best of our knowledge, no large studies have investigated the functions of islet beta cells and their correlations with liver dysfunction in NICCD patients. Therefore, the aims of this study were to explore the functions of islet beta cells and their correlations with liver dysfunction in NICCD patients.

2. Materials and methods

2.1. Subjects

From March to October 2013, a total of 36 NICCD patients (17 males and 19 females) were recruited for the NICCD group, and 50 subjects were recruited for the control group (23 males and 27 females). All subjects were infants aged less than 1 year.

2.1.1. Inclusion of case group.
NICCD was diagnosed on the basis of the presence of intrahepatic cholestasis, fatty liver, failure to thrive, hyperaminoacidemia, liver dysfunction, hypoglycemia, hypoproteinemia, and/or coagulation disorders,[1] and the diagnoses were confirmed by SLC25A13 gene analysis.[17]

2.1.2. Inclusion of control group.
Fifty infants who were selected as the control group were hospitalized with bronchitis but were treated and cured before leaving the hospital. Their liver functions were normal, they had no history of liver disease or family history of liver disease, and all the subjects had normal blood glucose, amino acid, and acylcarnitine profiles.

After NICCD diagnosis, the patients were treated with lactose (galactose)-restricted and medium-chain triacylglycerol (MCT)-supplemented formula, meropenem, gamma globulin, human serum albumin (HSA), supplementation with fat-soluble vitamins, and a low-carbohydrate, high-protein diet.[18,19]

3. Methods

3.1. SLC25Amutation analysis

Peripheral blood DNA from all subjects was prepared using a DNA extraction kit (Simgen Company, Hilden, Germany). DNA markers and the ordinary Taq and LA-Taq enzymes were purchased from TaKaRa Company (Takara Bio Inc., Beijing, China); the primers were synthesized by Thermo Fisher Scientific Co., Ltd. (Shanghai, China). The PCR reactions (PTC-200; Bio-Rad Life Science Inc., Berkeley, CA) and gel electrophoresis conditions (PAC300; Bio-Rad Life Science Inc., Berkeley, CA) were performed on the basis of previous studies.[9,20] Screening for 4 high-frequency SLC25A13 mutations (c.851_854del, c.1638_1660dup, IVS6+5G>A, and IVS16ins3kb) was performed in the NICCD group. In individuals in whom the initial screening revealed only 1 mutated allele, all 18 exons and their flanking sequences in the SLC25A13 gene were sequenced.

3.2. Detection of liver function

A 3 to 4-hour fast was required for all subjects before collection of venous blood samples. The following data were collected for liver function analyses: the total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), alanine aminotransferase (ALT), aspartate amino transferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), alpha-fetoprotein (AFP), albumin (ALB), and globulin (GLB) concentrations and the albumin/globulin ratio (A/G). Liver function analyses were completed by the biochemistry laboratory of the First Affiliated Hospital of Jinan University.

3.3. Evaluation of islet beta cell functions

Fasting blood glucose (FBG) levels were measured using the glucose oxidase method. Fasting insulin and C-peptide (C-P) were detected using chemiluminescence immunoassays. The homeostasis model of assessment for insulin resistance index (HOMA-IR) was evaluated using FBG (mmol/L) and fasting insulin (mU/L) as follows: HOMA-IR= FBG × fasting insulin/22.5.[21] In addition, the fasting beta cell function index (FBCI) was calculated to evaluate islet beta cell functions in the fasting state as follows: FBCI= fasting insulin/FBG.[21] Further, the HOMA beta cell function index (HBCI) was calculated to evaluate secretion by islet beta cells after glucose overload as follows: HBCI = 20 × fasting insulin/(FBG–3.5).[21]

3.4. Statistical analysis

The data were processed and analyzed using SPSS (version 16.0; SPSS Inc., Chicago, IL). Normally distributed data are expressed as the mean ± standard deviation (mean ± SD), and data with a skewed distribution are expressed as the median (with minimum and maximum values). The t test and Wilcoxon rank sum test were used to analyze the measured data, and the χ^2 test was used to analyze the relationships of FBG, fasting insulin, and C-P, as well as those of HOMA-IR, FBCI, and HBCI, with liver damage. P values < .05 were considered statistically significant.

3.5. Ethical statement

This study was approved by the Medical Ethical Committee of the First Affiliated Hospital at Jinan University in China and was conducted in accordance with the World Medical Association Declaration of Helsinki (WMADH 2008).

In this study, SLC25A13 genetic mutation analyses were conducted with written informed consent from every patient's guardian, following the approval of the First Affiliated Hospital of Jinan University.

4. Results

4.1. Patient demographics

In total, 36 patients (17 males and 19 females) with a definite diagnosis of NICCD were included in the NICCD group. Fifty individuals (23 males and 27 females) were included in the control group. The control group was younger than the NICCD group, but the difference did not reach statistical significance (Table 1).

4.2. Screening and Sequencing Results

Among the 36 NICCD patients in the study, 10 types of SLC25A13 mutations were detected. Four high-frequency mutations, including c.851_854del, c.1638_1660dup, IVS6+5G>A, and IVS16ins3kb, accounted for 61%, 11%, 6%, and 4%, respectively, with a sum of 82%. The other 6 mutations

Table 1

Liver functions and laboratory data of NICCD patients and normal control group.

Indices	NICCD group	Control group	Z	P
Gender (M/F)	36 (17/19)	50 (23/27)	—	—
Age, mo	6.5 ± 3.4[*]	6.2 ± 3.0[*]	0.43[‡]	.333
T-Bil, μmol/L	27.7 (1.4, 336.1)[†]	5.5 (1.1, 92.0)[†]	8.55	.005[§]
D-Bil, μmol/L	21. 4 (1.2, 238.7)[†]	1.9 (0.8, 26.1)[†]	10.64	.002[§]
I-Bil, μmol/L	7.1 (0.2, 132.3)[†]	3.8 (0.3, 70.6)[†]	3.75	.058
ALT, U/L	46.5 (11.0, 183.0)[†]	27.0 (13.0, 44.0)[†]	4.36	.000[‖]
AST, U/L	68.0 (25.0, 261.0)[†]	35.0 (26.0, 49.0)[†]	5.19	.000[‖]
GGT, U/L	127.0 (16.0, 447.0)[†]	24.0 (9.0, 154.0)[†]	4.60	.000[‖]
ALP, U/L	463.5 (213.0, 1087.0)[†]	315.0 (152.0, 570.0)[†]	3.29	.001[§]
AFP, ng/mL	2576.2 (5.0, 589,102.0)[§]	17.2 (1.7, 69.5)[†]	5.12	.000[‖]
ALB, g/L	40.59 ± 7.84[*]	44.03 ± 5.02[*]	2.31[‡]	.012[§]
GLB, g/L	17.01 ± .4.30[*]	25.20 ± 4.69[*]	8.27[‡]	.000[‖]
A/G	3.17 ± 1.12[*]	2.76 ± 0.74[*]	1.94[‡]	.029[§]

A/G = albumin/globulin, AFP = alpha-fetoprotein, ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate amino transferase, DBIL = direct bilirubin, GGT = gamma-glutamyl transpeptidase, GLB = globulin, IBIL = indirect bilirubin, NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency, TBIL = total bilirubin.
[*] Expressed as mean ± SD.
[†] Expressed as median (minimum, maximum).
[‡] Expressed as T values.
[§] $P < .05$.
[‖] $P < .001$.

were $IVSins6\,kb$, $R360X$, $C.2\,T > C$, $C.415\,G > A$, $Q259X$, and $IVS11+1\,G > A$.

4.3. Comparison of liver function parameters between the NICCD and control groups

The NICCD group had significantly higher TBIL, DBIL, ALT, AST, GGT, ALP and AFP levels, and A/G than the control group (Table 1), suggesting that the liver functions and metabolism were severely damaged in the NICCD patients. In addition, the NICCD patients had lower ALB and GLB levels than the control group.

4.4. Comparison of FBG, fasting insulin, C-P, and islet beta cell functions between the 2 groups

The differences in FBG, fasting insulin, C-P, HOMA-IR, FBCI, and HBCI between the NICCD and control groups were not significant ($P < .05$) (Table 2).

4.5. The correlations between FBG and fasting insulin and between FBG and C–P in NICCD patients

A linear correlation was found between FBG and fasting insulin and between FBG and C-P in the NICCD patients. Positive correlations were observed between FBG and fasting insulin ($t = 0.24$, $P < .001$, Fig. 1) and between FBG and C-P ($t = 3.55$, $P = .001$, Fig. 2). The determination coefficients for these correlations were 0.352 and 0.277, respectively.

4.6. Correlation analysis between FBG, fasting insulin, and C-P and liver damage in NICCD patients

In the NICCD group (fasting insulin and C-P were absent in 1 patient), the χ^2 test was used to assess the correlations of liver damage with FBG, fasting insulin, C-P, HOMA-IR, FBCI, HBCI, FBG. Fasting insulin and C-P, and the ALT, AST, GGT, ALP, and AFP liver function indices were divided into 2 or 3 grades on the basis of the normal ranges[22] and the data characteristics in the study.

The FBG level was not related to the ALT, AST, GGT, ALP, or AFP levels ($P > .05$, Table 3). A linear trend was observed for the correlation of the ALT level with the fasting insulin level ($\chi^2 = 5.15$, $P = .023$); the Spearman correlation coefficient was 0.425 ($P = .011$). This trend suggests that the fasting insulin level increases with increasing ALT concentration. However, the fasting insulin level was not related to elevation of the other liver function indices ($P > .05$, Table 3). Further, the C-P level was not associated with the degree of elevation of ALT, AST, GGT, ALP, or AFP ($P > .05$, Table 3).

Table 2

Islet beta cell functions between NICCD patients and normal control group.

Indices	NICCD group	Control group	Z	P
FBG, mmol/L	5.00 ± 1.20[*]	5.28 ± 0.68[*]	1.26[‡]	.106
Fasting insulin, mU/L	4.72 (0.50, 72.31)[†]	3.51 (1.62, 37.21)[†]	0.63	.529
C-P, ng/mL	1.17 (0.10, 6.64)[†]	0.84 (0.42, 3.84)[†]	1.30	.195
HOMA-IR	1.13 (0.03, 24.78)[†]	0.80 (0.39, 10.93)[†]	0.69	.487
FBCI	0.87 (0.12, 11.90)[†]	0.73 (0.23, 5.63)[†]	0.60	.548
HBCI	56.36 (−76.92, 614.75)[†]	50.32 (8.55, 239.29)[†]	0.35	.728

C-P = C-peptide, FBCI = fasting beta cell function, FBG = fasting sblood glucose, HBCI = the HOMA beta cell function index, HOMA-IR = the homeostasis model of assessment for the insulin resistant index, NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency.
[*] Expressed as mean ± SD.
[†] Expressed as median (minimum, maximum).
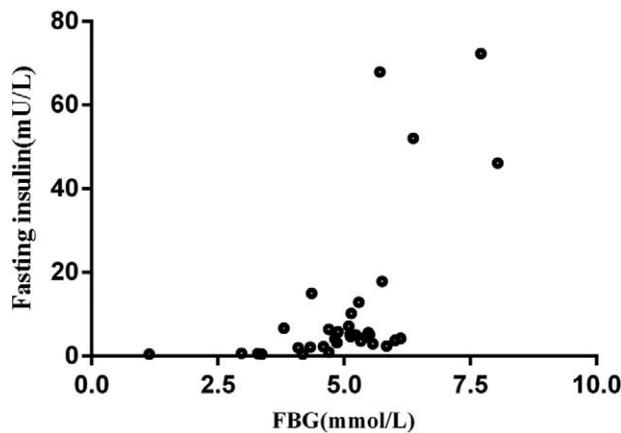[‡] Expressed as T values.

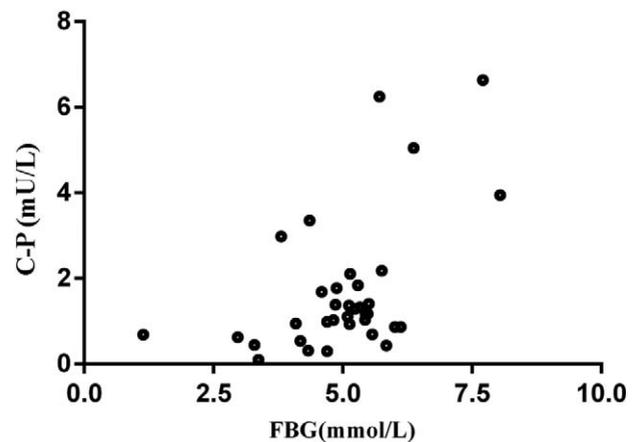Figure 1. A positive correlation was found between FBG and fasting insulin (t = 0.24, P < .001).

Figure 2. A positive correlation was found between FBG and C-P (t = 3.55, P = .001).

4.7. Correlation analysis of HOMA-IR, FBCI, and HBCI with liver damage in NICCD patients

HOMA-IR, FBCI, and HBCI were divided into 3 levels according to the distribution of the quartile characteristics and the horizontal magnitude.

On the basis of the Chi-square test, the ALT and HOMA-IR levels exhibited a linear variation trend with a significant linear regression component (χ^2 = 5.15, P = .023), with rank correlation coefficient of 0.425 (P = .011). This analysis suggests that the HOMA-IR level increases with increasing ALT concentration. However, the HOMA-IR level was not related to the elevations in the other liver function indices (P > .05, Table 4).

The ALT and HBCI levels exhibited linear variation trends in the Chi-square test, and the linear regression component was significant (χ^2 = 3.87, P = .049), with the rank correlation coefficient of 0.368 (P = .032). This analysis suggests that FBCI level increases with increasing ALT concentration. However, the FBCI level was not related to the elevations in the other liver function indices (P > .05, Table 4).

On the basis of a Chi-squared test, the ALT and FBCI levels exhibited linear variation trends with significant linear regression

components in the Chi-square test (χ^2 = 3.90, P = .048), with a rank correlation coefficient of 0.360 (P = .034). This analysis suggests HBCI level increases with increasing ALT concentration. However, the HBCI level was not related to the elevations in the other liver function indices (P > .05, Table 4).

5. Discussion

Citrin is an aspartate-glutamate carrier that is mitochondrial transport protein encoded by the gene *SLC25A13* on chromosome 7q21.3,[8,23,24] and its deficiency causes CD.[17] CD is one of the most common classical inborn errors in the metabolism of amino acids and organic acids and the oxidation of fatty acids in Eastern Asia, including China.[25] The functions of islet beta cells and their correlations with hepatic dysfunction in NICCD patients are not fully understood. Although differences in some indices between NICCD and non-NICCD patients have been reported,[1,26,27] the absence of a "true" control group in these studies has led to a vague interpretation of the results. In the present study, a control group of healthy infants was used to unravel the association between NICCD and some biochemical abnormalities.

Table 3

Correlation analysis of FBG, fasting insulin, and C-P with liver dysfunction in NICCD patients (n = 35).

Indices	Range	The range of FBG, mmol/L					The range of fasting insulin, mU/L					The range of C-P, ng/mL				
		< 2.80	2.80–5.60	> 5.60	χ^2	P	0.50–4.72	4.73–7.12	> 7.12	χ^2	P	0.10–0.87	0.88–1.84	> 1.84	χ^2	P
ALT, U/L	<30	0	4	2			4	1	1			3	2	1		
	30–65	1	14	2	0.17	.681	12	3	2	5.15	.023*	6	9	2	3.25	.071
	>65	0	8	4			2	5	5			2	5	5		
AST, U/L	<37	1	2	1			1	2	1			1	2	1		
	37–68	0	10	3	0.39	.531	11	1	1	1.43	.231	6	6	1	1.13	.287
	>68	0	14	4			6	6	6			4	8	6		
GGT (U/L)	12–123	0	14	3			9	5	3			5	8	4		
	>123	1	12	5	0.08	.775	9	4	5	0.22	.639	6	8	4	0.04	.835
ALP, U/L	185–555	1	17	6			14	5	5			10	10	4		
	>555	0	9	2	0.02	.878	4	4	3	0.89	.344	1	6	4	3.74	.053
AFP, ng/mL	<88	0	8	1			6	3	0			3	6	0		
	88–2576.2	0	5	3	0.01	.932	4	2	2	2.57	.109	3	4	1	2.45	.118
	>2576.2	1	13	4			8	4	6			5	6	7		

AFP = alpha-fetoprotein, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate amino transferase, C-P = C-peptide, FBG = fasting blood glucose, GGT = gamma-glutamyl transpeptidase, NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency.
* P < .05.

Table 4

Correlation analysis of HOMA-IR, FBCI, HBCI with liver dysfunction in NICCD patients (n=35).

Indices	Range	The range of HOMA-IR			χ²	P	The range of FBCI			χ²	P	The range of HBCI			χ²	P
		< 1.13	1.14–1.79	> 1.79			< 0.90	0.91–1.80	> 1.80			< 57.13	57.14–128.88	> 128.88		
ALT, U/L	<30	4	1	1			4	1	1			3	2	1		
	30–65	12	3	2	5.15	.023*	11	3	2	3.87	.049*	6	9	2	3.90	.048*
	>65	2	5	5			3	4	5			2	5	5		
AST, U/L	<37	1	2	1			1	2	1			1	2	1		
	37–68	11	1	1	1.43	.231	11	1	1	1.43	.231	6	6	1	0.81	.369
	>68	6	6	6			6	6	6			4	8	6		
GGT, U/L	12–123	10	4	3			9	5	3			5	8	4		
	>123	8	5	5	0.77	.380	9	4	5	0.22	.640	6	8	4	0.01	.953
ALP, U/L	185–555	13	6	5			14	5	5			10	10	4		
	>555	5	3	3	0.25	.614	4	4	3	0.89	.344	1	6	4	1.92	.165
AFP, ng/mL	<88	6	3	0			6	3	0			3	6	0		
	88–2576.2	3	3	2	1.85	.174	5	1	2	0.55	.460	3	4	1	3.41	.065
	>2576.2	9	3	6			7	5	6			5	6	7		

AFP = alpha-fetoprotein, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate amino transferase, FBCI = fasting beta cell function, GGT = gamma-glutamyl transpeptidase, HBCI = the HOMA beta cell function index, HOMA-IR = the homeostasis model of assessment for the insulin resistant index, NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency.

* $P < .05$.

5.1. Liver dysfunction in NICCD patients

Impaired liver cell function leads to abnormal liver synthesis and secretion, resulting in cholestasis. NICCD patients manifest cholestatic jaundice, accompanied by liver dysfunction, hepatic steatosis, and biochemical metabolic disorders. In the present study, NICCD group presented with higher TBIL, DBIL, ALT, AST, GGT, ALP, and AFP levels; it indicated that the liver dysfunction and biliary obstruction were occurred in NICCD patients. The AST levels were higher than the ALT levels, which was consistent with previous studies.[16,26] ALT is commonly used to evaluate liver function and mainly expressed in the liver, followed by the skeletal muscle, kidney, myocardium, and other tissues. In liver cells, ALT is mainly located outside of the mitochondria; when liver cells are damaged, the permeability of the liver cells increases, and ALT in the cytoplasm is released into the blood. Unlike ALT, AST is mainly present within the mitochondria. When liver cells suffer moderate damage, ALT leakage is greater than AST leakage; when liver cells are seriously damaged, the mitochondrial membrane is also damaged, and AST is significantly increased. Further, the protein synthesis may be affected by liver dysfunction. In the present study, lower ALB and GLB levels were observed in the NICCD patients.

5.2. The functions of islet beta cells in NICCD patients

Previous case studies have indicated that hypoglycemia is associated with NICCD.[16,19,28] Some scholars speculated that hypoglycemia was caused by a disturbance in gluconeogenesis because the aspartate–glutamate carrier citrin provided substrates for gluconeogenesis as a part of the pathway for the conversion of amino acids to glucose.[28–30] In the present study, no significant differences in FBG, fasting insulin, C-P, HOMA-IR, FBCI, or HBCI were observed between the NICCD and control groups. Hypoglycemia was detected in 1 patient, and the incidence of hypoglycemia was 3%. Further, we investigated the correlations between FBG and fasting insulin and between FBG and C-P in the NICCD patients by linear correlation analysis. A linear relationship was found between FBG and fasting insulin and between FBG and C-P, with determination coefficients of 0.352 and 0.277, respectively. These coefficients suggested that the correlations were low. Insulin-lowering blood glucose depends on the body's sensitivity to insulin with better islet beta cell functions leading to better sensitivity of the body to insulin. In the fasting state, blood glucose, insulin, and insulin sensitivity are in a steady state.[31] The HOMA-IR, FBCI, and HBCI are calculated from the blood glucose and insulin levels under basic conditions[21] and mainly reflect the insulin sensitivity of the liver and kidney tissues.[32] As a result, these measures can be used to evaluate islet beta cell functions. The results of this study demonstrated that there was no difference in islet beta cell functions between the NICCD patients and the control group.

5.3. Correlation between islet beta cell functions and liver dysfunction

In this study, fasting insulin, HOMA-IR, FBCI, and HBCI were positively correlated with the increased ALT level. These correlations indicated that fasting insulin, HOMA-IR, FBCI, and HBCI were also increased with the increased ALT level. Therefore, the liver dysfunction may be correlated with islet beta cell functions in NICCD patients.

Under normal circumstances, 50% to 80% of insulin is cleared by the liver. Chronic liver disease can reduce the number of liver cells and lead to liver dysfunction, causing reduced insulin inactivation. If cirrhosis occurs, liver uptake of insulin is further reduced, eventually leading to hyperinsulinemia.[33] A previous study made the following assumptions[34]: a high level of insulin can promote the proliferation of hepatic stellate cells, hyperglycemia can promote the expression of tissue factors, activation of hepatic stellate cells is the central link to liver fibrosis, and the expression of tissue growth factors can promote the liver fibrosis process.

Some previous studies[19,35,36] indicated that NICCD patients' liver biopsies showed varying degrees of hepatic steatosis, diffuse hepatocyte microbubble degeneration, or bile duct dilatation as well as mild to moderate liver fibrosis. These conditions may be associated with the relatively high levels of insulin in children with NICCD. When chronic liver disease or liver fibrosis occurs, insulin secretion and metabolism are abnormal, leading to deterioration of liver function. The process mainly consists of 3 stages.[37] First, early in the disease, there is no barrier for insulin secretion and synthesis. Due to liver disorders, a reduction of liver cells occurs, and a portacaval shunt is needed. The liver uptake and insulin inactivation functions are reduced, and most patients

present an increased insulin level. Otherwise, glucose tolerance can be normal at this stage due to the increased levels of insulin antagonistic substances. Second, IR gradually increases with the progression of the disease, and islet beta cells cannot adequately account for the increased secretion of insulin, which leads to a relative lack of insulin secretion. As a result, glucose tolerance becomes impaired. Third, the long-term compensatory secretion of insulin leads to islet beta cell failure and an absolute lack of insulin secretion until the patients ultimately develop hepatogenic diabetes. Consequently, we speculate that NICCD patients with liver dysfunction may be in the early stage of chronic liver damage. At this time, the damage to liver cells may be less severe. This assessment is consistent with the evaluation of liver biopsies in NICCD children with mild to severe hepatic fibrosis.

6. Conclusion

This work presented liver dysfunction in NICCD patients and showed that the AST levels were higher than the ALT levels. Moreover, fasting insulin, HOMA-IR, FBCI, and HBCI were increased with increases in the ALT level. We speculate that the liver dysfunction may be correlated with islet beta cell functions in NICCD patients. The main limitation of this study was its retrospective nature. Some tests were not available from this study. Thus, a more comprehensive evaluation of the liver function of NICCD patients is needed in the future.

Acknowledgments

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