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Original Article

Overdiagnosis of adrenal insufficiency in children with biliary atresia

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Highlights

- Children with biliary atresia (BA) frequently have reduced cortisol-binding globulin levels. The prevalence of adrenal insufficiency (AI), diagnosed based on peak serum total cortisol levels, was overestimated in these patients.
- The free cortisol index (FCI) and calculated free cortisol (cFC) levels could help reduce the overdiagnosis of AI in patients with BA.

Abstract. Serum cortisol mainly binds to the cortisol-binding globulin (CBG). Children with biliary atresia (BA) may have low serum CBG levels; thus, low serum total cortisol (TC) levels and adrenal insufficiency (AI) may be overdiagnosed. This study aimed to assess adrenal function in children with BA. All the patients underwent adrenocorticotropic hormone (ACTH) stimulation tests. Plasma ACTH, serum TC, and CBG levels were measured at baseline, with additional TC measurements at 30 and 60 min during testing. Free cortisol (FC) index (FCI) and calculated FC (cFC) were also calculated. AI was defined as peak TC <500 nmol/L (<18 μg/dL), peak FCI <12 nmol/mg, or peak cFC <33 nmol/L (<1.2 μg/dL). This study enrolled 71 children with BA. The Median (IQR) age of the patients was 5.5 (1.7–11.4) years. Twenty-five (35%) patients were diagnosed with AI based on the peak TC. In the AI group, the median serum CBG level was significantly lower than that in the non-AI group (481 *vs.* 533 nmol/L, $p = 0.03$). Only eight patients (11%) met all three AI criteria (six secondary AI and two primary AI). In conclusion, low serum CBG levels contribute to reduced peak TC and, consequently, overdiagnosing AI. Peak FCI and cFC could help reduce the overdiagnosis of AI.

Key words: adrenal insufficiency, cirrhosis, cortisol binding globulin, free cortisol

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Introduction

Cortisol is a steroid hormone secreted from the adrenal cortex under the control of the hypothalamic-pituitary-adrenal (HPA) axis. Approximately 90% of total serum cortisol (TC) is protein bound. The main cortisol-binding protein is cortisol-binding globulin (CBG). Unbound cortisol or free cortisol (FC), which is biologically active, accounts for only 10% of TC (1). The TC (not FC) is generally measured in clinical practice. Therefore, changes in serum CBG levels affect the measured serum TC concentration, whereas the serum FC concentration remains unchanged (2).

The inadequate production or action of cortisol under physiological and/or pathological conditions causes adrenal insufficiency (AI). Several adult studies have reported a high prevalence of AI in patients with both compensated and decompensated cirrhosis and in both early and late post-liver transplantation (3, 4). "Hepatoadrenal syndrome" has been proposed as the mechanism underlying AI in these patients (5–9). A recent study in children with cirrhosis demonstrated that the prevalence of AI was 54% and that AI was associated with cirrhosis complications (10).

However, data on the adrenal function in children with cirrhosis are scanty (10). To the best of our knowledge, adrenal function assessments, specifically in children with biliary atresia (BA) have not yet been reported. Several mechanisms have been proposed to explain the impaired adrenal function in patients with cirrhosis. Elevated levels of pro-inflammatory cytokines can inhibit HPA axis function (4, 11–15). Moreover, some patients previously treated with long-term glucocorticoids following liver transplantation may exhibit HPA axis suppression secondary to exogenous glucocorticoids (8, 16). Additionally, CBG levels may be low in patients with cirrhosis because of impaired synthetic liver function. Therefore, a decrease in CBG-bound cortisol could lead to low serum TC levels, despite normal serum FC. The prevalence of AI is overestimated (17–21). Theoretically, serum FC is more appropriate than serum TC in this condition. However, serum FC measurement is relatively expensive and time-consuming and is usually performed in research settings (17, 20, 22). Calculated FC (cFC) and FC index (FCI), which are indirect estimates of FC, have been proposed as good surrogate markers for measured FC (19, 21, 23, 24).

Biliary atresia (BA) is a common cause of neonatal-onset progressive obstructive cholangiopathy that leads to progressive liver fibrosis. Without timely treatment with Kasai portoenterostomy in patients with BA, cirrhosis eventually occurs and liver transplantation is required. This condition is the most common indication for pediatric liver transplantation (25). This study aimed to evaluate the adrenal function in children with BA using both serum TC and surrogate markers of serum FC.

Materials and Methods

Patients

This cross-sectional study was conducted at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand between May 2020 and October 2021. This study enrolled hemodynamically stable patients with BA aged 1–20 yr. BA was diagnosed based on clinical and laboratory findings and typical imaging and/or histopathological findings. Data related to BA were obtained from the medical records. Patients with preexisting HPA axis dysfunction, exposure to glucocorticoids or drugs that interfere with the HPA axis, or CBG synthesis within one year prior to the study were excluded. All patients had clinical and biochemical findings consistent with those of hemodynamically stable cirrhosis (normal vital signs and no acute critical illness). Written informed consent was obtained from patients and their legal guardians. This study was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (MURA 2020/511).

All patients underwent clinical and biochemical evaluations and adrenal function testing. Clinical data were collected from the medical records at the time of blood testing. These included anthropometry, history of Kasai portoenterostomy, history of liver transplantation (LT), present and past illnesses (e.g., history of cholangitis, ascites, spontaneous bacterial peritonitis, variceal bleeding, and hepatic encephalopathy), Pediatric End-Stage Liver Disease (PELD) score, history of glucocorticoid use, and biochemical parameters (complete blood count, liver function tests, coagulation, serum creatinine, total cholesterol, and electrolytes). Cirrhosis was diagnosed in patients with evidence of chronic liver disease (jaundice, palmar erythema, and spider nevi) and signs of portal hypertension (splenomegaly, ascites, esophageal varices, and impaired liver synthetic function). The PELD score is used to estimate the severity of chronic liver disease in children without LT and to predict 3-month mortality in children awaiting LT (e.g., scores > 20 and > 30, 3-month mortality rates were approximately 20% and 30–40%, respectively) (26, 27).

Adrenal function assessment and CBG measurement

Adrenocorticotropic hormone (ACTH) stimulation test was performed using 1 μg synthetic ACTH (Cortrosyn®). Blood samples for serum TC, albumin, CBG, and plasma ACTH levels were collected at baseline (0 min). Intravenous 1 μg of ACTH was administered and then blood samples for serum TC were collected at 30 and 60 min after the ACTH injection.

cFC and FCI were determined from the functions of TC and CBG, as shown in the formulas below.

AI was defined as a peak TC level of < 500 nmol/L

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(< 18 µg/dL) (28, 29). In conditions with altered cortisol binding proteins, either peak cFC < 33 nmol/L (< 1.2 µg/dL) (30, 31) or peak FCI < 0.7 nmol/nmol (< 12 nmol/mg) (22, 23) was used for diagnosing AI. Primary AI was differentiated from secondary AI by elevated basal morning plasma ACTH levels > 22 pmol/L (> 100 pg/mL) (> 2-fold the upper limit of the reference range) (29).

Serum CBG was measured by using a commercial ELISA kit (BioVender, Czech Republic; a detection limit of 15 nmol/L (1.0 µg/mL), an assay CV < 6%). Serum TC was measured by using an electrochemiluminescence immunoassay on a Roche modular E170 analyzer (Roche Diagnostics, Mannheim, Germany; a detection limit of 2.75 nmol/L (1.0 µg/dL), an assay CV < 5%). Plasma ACTH was measured by using a chemiluminescence immunoassay on an Immulite 2500 analyzer (Siemens Medical Solutions Diagnostics Limited, Llanberis, UK; a detection limit of 1.1 pmol/L (5 pg/mL), an assay CV < 6%).

Calculations

FCI = serum TC/CBG.

cFC (Coolens equation) = $\sqrt{Z^2 + 0.0122 \times TC} - Z$, where Z = 0.0167 + 0.182 × (CBG-TC) (30).

PELD = 0.436 × [age (<1 y)] – 0.687 × Log_e (albumin g/dL) + 0.480 × Log_e (TB mg/dL) + 1.87 × Log_e (INR) + 0.667 (growth failure (< –2 standard deviation (SD) present), where TB = total bilirubin and INR = international normalized ratio.

Statistical analysis

Data are presented as median (interquartile range, IQR) or frequency (percentage), as appropriate. For comparisons between two groups, the Chi-squared or Fisher's exact test was used for categorical variables, and the Student's *t*-test or Mann-Whitney U test was used for continuous variables. Correlation analysis between continuous variables was performed using Spearman's correlation coefficient. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the SPSS statistical package (SPSS, Chicago, IL) version 22.0.

Results

A total of 71 hemodynamically stable children with BA were enrolled in this study. There were 44 non-liver transplantation (non-LT) patients and 27 LT patients. Their median (IQR) age was 5.5 (1.7–11.4) yr. Forty patients (56%) were female. The clinical characteristics of the patients are shown in **Table 1**.

Assessment of adrenal function

Of the 71 patients, 25 (35%) had AI defined by the peak TC criterion (peak TC < 500 nmol/L or < 18 µg/dL), 14 of 44 (32%) in the non-LT group, and 11 of 27 (41%) in the LT group. The stimulated cortisol responses to the ACTH test (peak TC, FCI, and cFC) of patients in the non-LT and LT groups were not significantly different (data not shown). Therefore, we analyzed all 71 patients in the entire group. All patients were divided into two groups based on their adrenal function, using the peak TC criterion, AI, and normal adrenal function (NAF) (**Table 1**). No significant differences in most clinical and biochemical findings were observed between the two groups. Patients in the AI group were significantly older than those in the NAF group [7.8 (4.5–12.8) *vs.* 4.6 (1.4–8.1) years, $p = 0.02$].

Basal serum TC and CBG levels were significantly

Table 1. Baseline clinical characteristics, hormonal and biochemical profiles of patients, comparing between adrenal insufficiency (AI) and normal adrenal function (NAF) groups

	All patients (n = 71)	AI (n = 25)	NAF (n = 46)	*p*-value
Age (yr)*	5.5 (1.7–11.4)	7.8 (4.5–12.8)	4.6 (1.4–8.1)	**0.02**
Sex (M/F), n (%)	31 (44)/40 (56)	10 (40)/15 (60)	21 (46)/25 (54)	0.64
LT/Non-LT, n (%)	27 (38)/44 (62)	11 (44)/14 (56)	15 (35)/30 (65)	0.45
Albumin (g/L)*	34.2 (27.2–40.6)	34.8 (28.8–40.1)	33.8 (26.5–40.9)	0.68
Total cholesterol (mmol/L)*	4.3 (3.4–5.2)	4.6 (3.6–5.2)	4.1 (3.2–5.2)	0.33
CBG (nmol/L)*	515 (412–636)	481 (275–601)	533 (447–670)	**0.03**
ACTH (pmol/L)*	4.2 (2.9–6.6)	3.7 (2.6–5.2)	4.4 (2.9–6.8)	0.45
Basal TC (nmol/L)*	294 (184–420)	225 (135–299)	349 (231–519)	**< 0.01**
Basal FCI (nmol/nmol)*	0.57 (0.36–0.87)	0.48 (0.29–0.64)	0.61 (0.41–1.10)	0.05
Basal cFC (nmol/L)*	22.0 (11.0–43.9)	19.2 (8.2–24.7)	24.7 (13.7–57.6)	**0.01**
Peak TC (nmol/L)*	560 (442–667)	414 (332–453)	626 (565–771)	**< 0.01**
Peak FCI (nmol/nmol)*	1.08 (0.88–1.45)	0.90 (0.61–1.14)	1.15 (0.94–1.63)	**< 0.01**
Peak cFC (nmol/L)*	63.1 (43.9–93.3)	43.9 (24.7–54.9)	74.1 (57.6–112.5)	**< 0.01**

*Data are expressed as median (IQR); otherwise, as indicated. LT, liver transplantation; ACTH, adrenocorticotropic hormone (reference range:1.3–10.6 pmol/L); CBG, cortisol binding globulin (reference range:653–890 nmol/L); TC, total cortisol; FCI, free cortisol index; cFC, calculated free cortisol (Conversion factor: ACTH, pmol/L × 4.5 = pg/mL; CBG, nmol/L × 0.058 = µg/mL; TC, nmol/L × 0.036 = µg/dL; FCI, nmol/nmol × 17.14 = nmol/mg; cFC, nmol/L × 0.036 = µg/dL).

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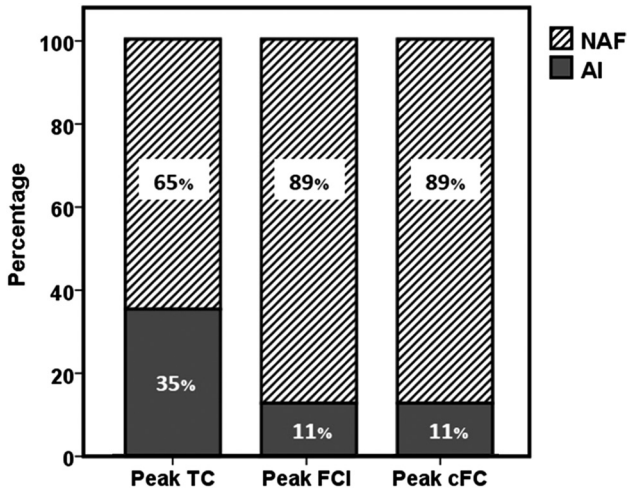

Fig. 1. Percentage of patients (n = 71) with adrenal insufficiency (AI) (grey bars) and normal adrenal function (NAF) (striped bars) diagnosed by peak serum total cortisol (TC) (< 500 nmol/L), peak free cortisol index (FCI) (< 0.7 nmol/nmol) and peak calculated free cortisol (cFC) (< 33 nmol/L).

lower in the AI group than those in the NAF group. However, when basal TC was corrected for serum CBG, as represented by calculating the FCI, the basal FCI was not significantly different between the AI and NAF groups ($p = 0.05$) (**Table 1**). Twenty-five patients (35%) were diagnosed with AI based on the peak TC criterion, whereas only eight patients (11%) were diagnosed with AI based on either the peak FCI or peak cFC criterion (**Fig. 1**).

Both serum CBG and basal serum TC levels positively correlated with peak TC ($r = 0.33$, $p < 0.01$, and $r = 0.69$, $p < 0.01$, respectively) (**Figs. 2A and 2B**). Peak FCI and cFC were significantly correlated with peak TC ($r = 0.70$, $p < 0.01$ and $r = 0.86$, $p < 0.01$, respectively) (**Figs. 2C and 2D**).

Eight patients met all three AI criteria of AI by peak TC, FCI, and cFC (**Figs. 2C and 2D**) (**Table 2**). Six of the eight index patients were in the non-LT group. Six patients developed secondary AI. The remaining 2 patients had primary AI (elevated plasma ACTH, > 22 pmol/L or > 100 pg/mL, and low stimulated serum cortisol), presumably secondary to adrenal damage

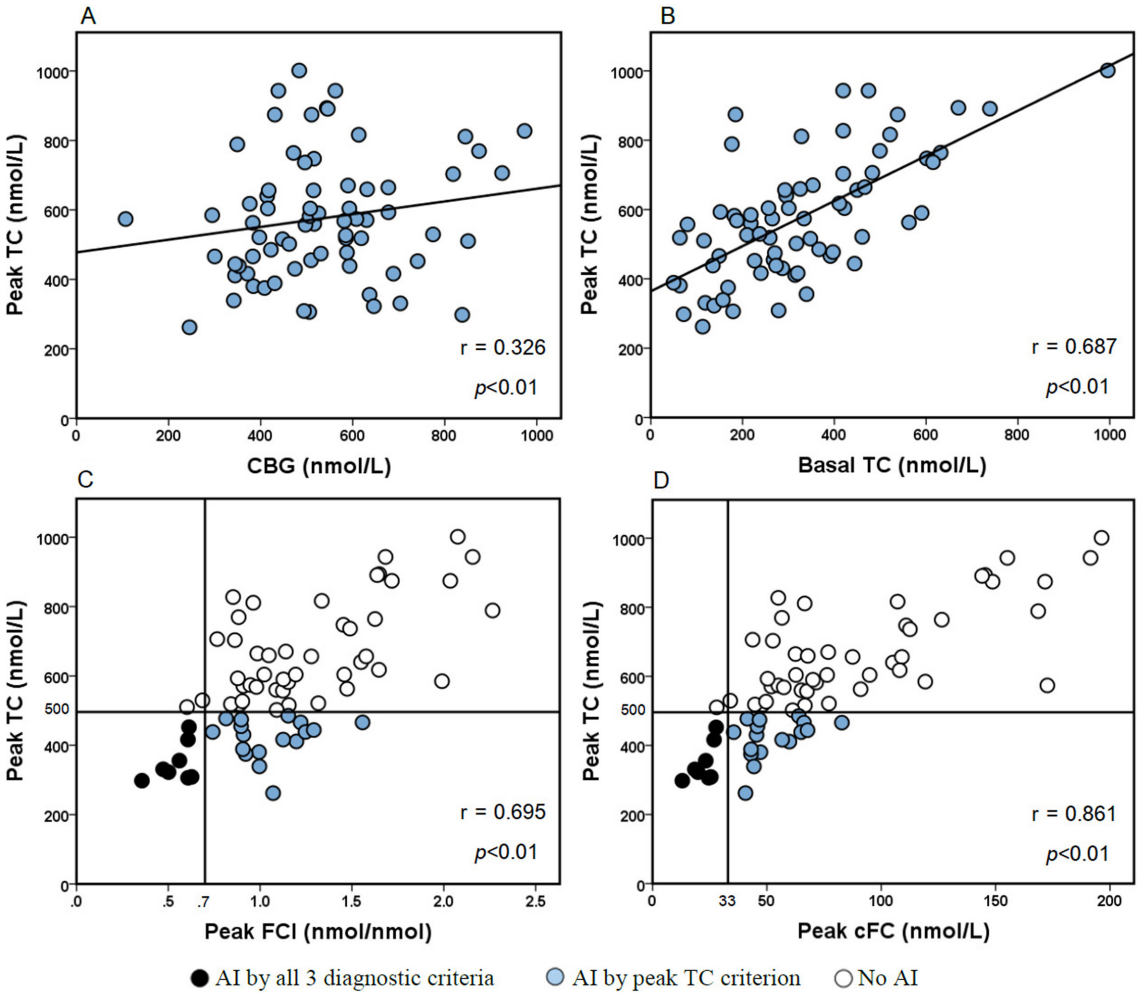

Fig. 2. Correlation between peak serum total cortisol (TC) and cortisol binding globulin (CBG) (A), peak TC and basal TC (B), peak TC and peak free cortisol index (FCI) (C), and peak TC and peak calculated free cortisol (cFC) (D). Horizontal lines represent peak TC cut-off (500 nmol/L) (C,D). Vertical lines represent peak FCI cut-off (0.7 nmol/nmol) (C) and peak cFC (33 nmol/L) (D).

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from septic shock and hemorrhagic shock (**Table 2**, patients 1 and 2). Patient 1, who had gram-negative septicemia with septic shock and two episodes of severe intestinal bleeding following LT, underwent ACTH testing two years after the episode. Patient 2 had gram-negative septicemia with septic shock and disseminated intravascular coagulation at the age of 4 mo, and ACTH testing was performed 3 years after the episode. However, adrenal imaging was not performed due to biochemical subclinical adrenal insufficiency.

Adrenal function and severity of liver disease

Regarding the association between the severity of liver disease in non-LT patients and adrenal function, the non-LT patients were divided into two subgroups according to the PELD score: PELD score 0–9 (n = 24) and PELD score > 9 (n = 20). The higher PELD score group, in which patients had more severe liver disease, tended to have lower serum CBG [443 (414–605) *vs.* 624 (442–766) nmol/L] or [25.8 (24.1–35.2) *vs.* 36.3 (25.7–44.6) μg/mL (p = 0.09)] (Supplementary Table 1). There was a negative correlation between serum CBG and PELD scores (r = –0.38, p <0.01) (**Fig. 3A**). Although the higher PELD score group had lower serum CBG, their basal TC concentrations were higher than those of the lower PELD score group [340 (200–456) *vs.* 217 (140–324) nmol/L] or [12.4 (7.3–16.6) *vs.* 7.9 (5.1–11.8) μg/dL (p = 0.03)] while their peak TC were not significantly different [587 (478–820) *vs.* 519 (417–675) nmol/L] or [21.4 (17.4–29.9) *vs.* 18.9 (15.2–24.6) μg/dL (p = 0.17)]. Nevertheless, peak FCI and peak cFC in the higher PELD score group were significantly higher than those of the lower PELD score group [1.32 (0.93–1.68) *vs.* 0.90 (0.63–1.12) nmol/nmol (p < 0.01)] or [22.7 (15.9–28.8) *vs.* 15.4 (10.8–19.2) nmol/mg], and [79.3 (54.6–138.0) *vs.* 50.7 (29.4–64.8) nmol/L, (p <0.01)] or [2.89 (1.99–5.03) *vs.* 1.85 (1.07–2.36) μg/dL], respectively] (Supplementary Table 1). In addition, the PELD score was positively correlated with both peak FCI (r = 0.70, p < 0.01) and peak cFC (r = 0.41, p < 0.01) (**Figs. 3B and 3C**) but not with peak TC (**Fig. 3D**).

Discussion

The present study found that the prevalence of AI diagnosed by a peak TC < 500 nmol/L (< 18 μg/dL) was 35%, which is in agreement with previous studies (7, 10, 17). Using either peak FCI or cFC criteria, the prevalence of AI was 11%, which is comparable to that of previous reports in adult patients (17, 22).

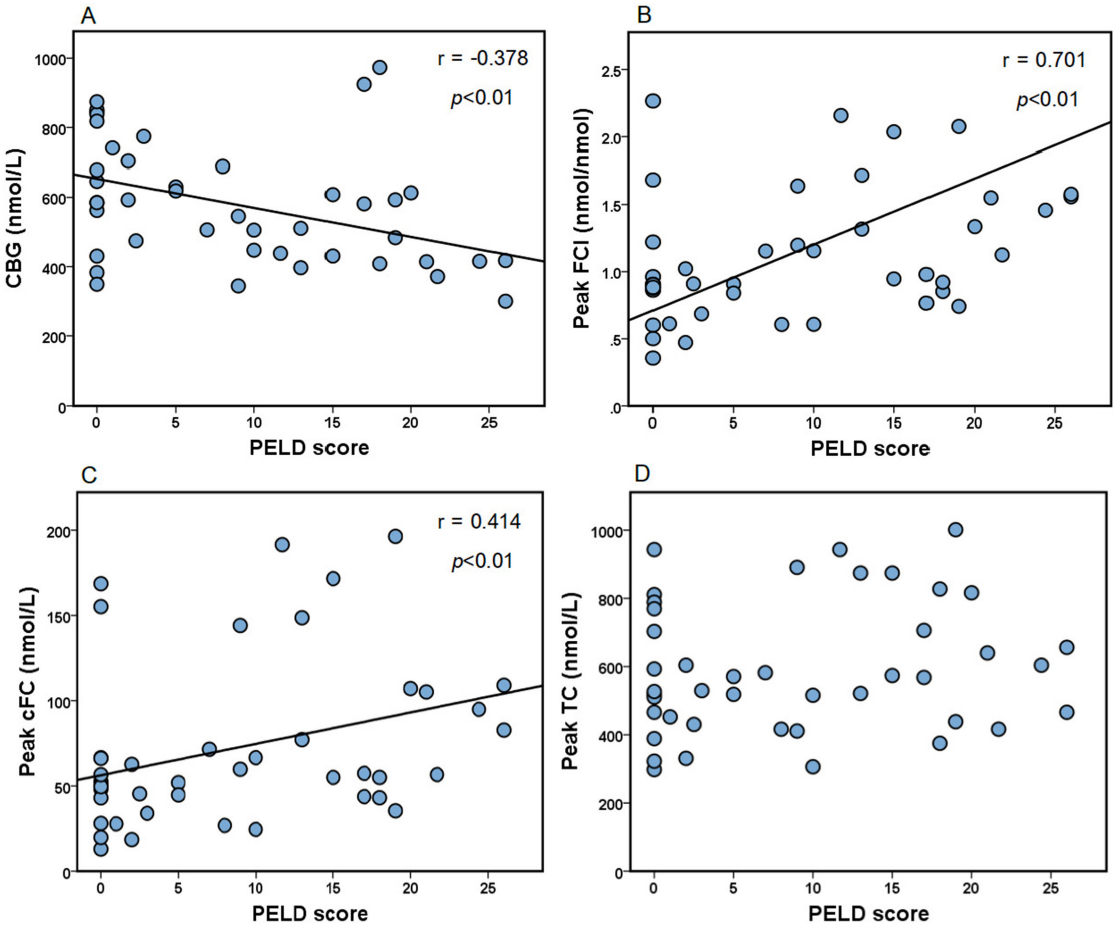

Fig. 3. Correlation between serum cortisol binding globulin (CBG) and Pediatric End-Stage Liver disease (PELD) score (A), peak serum free cortisol index (FCI) and PELD score (B), peak serum calculated free cortisol (cFC) and PELD score (C), no correlation between peak serum total cortisol (TC) and PELD score (D).

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Table 2. Characteristics of 8 index cases with adrenal insufficiency (AI) ** compared with those in the NAF and the AI (by peak TC) groups

Patient No.	1 (LT)	2	3	4	5	6	7	8 (LT)	AI by peak TC, n=17	NAF, n = 46
Age (yr)	5	3.5	12	11	4.5	0.1	1.5	11	9.2 (5.9–14.5)*	4.6 (1.4–8.1)*
Sex (M/F), n (%)	M	M	F	M	F	M	M	F	5 (29)/12(71)	21 (46)/25 (54)
PELD score	NA	1	2	0	0	10	8	NA	13.5 (0–21)*, n = 8	9.5 (0–17)*, n = 30
ACTH (pmol/L)	30.6	34.8	3.9	2.2	2.7	4.4	2.2	5.7	3.7 (2.8–4.9)*	4.4 (2.9–6.8)*
CBG (nmol/L)	637	741	703	838	646	505	689	493	428 (272–588)*	533 (447–670)*
Albumin (g/L)	34.8	31.0	26.3	37.4	32.9	25.9	23.9	42.8	36.3 (29.5–41.2)*	33.8 (26.5–40.9)*
Basal TC (nmol/L)	338	225	118	19	137	178	318	277	227 (142–301)*	349 (231–519)*
Peak TC (nmol/L)	354	450	329	296	321	305	414	307	421 (385–466)*	626 (565–771)*
Peak FCI (nmol/nmol)	0.56	0.61	0.47	0.36	0.50	0.61	0.61	0.62	0.99 (0.90–1.20)*	1.15 (0.94–1.63)*
Peak cFC (nmol/L)	23.3	27.7	18.4	12.9	19.8	24.4	26.6	25.2	46.7 (43.1–61.9)*	74.1 (57.6–112.5)*

*Data are expressed as median (IQR); otherwise, as indicated. **AI was defined as having all 3 criteria: peak TC < 500 nmol/L, peak FCI < 0.7 nmol/nmol, and peak cFC < 33 nmol/L. LT, liver transplantation; NAF, normal adrenal function; TC, total cortisol; FCI, free cortisol index; cFC, calculated free cortisol; ACTH, adrenocorticotropic hormone (reference range: 1.3–10.6 pmol/L), CBG, cortisol binding globulin (reference range: 653–890 nmol/L); PELD, Pediatric End-Stage Liver Disease; N, not applicable (conversion factor: ACTH, pmol/L × 4.5 = pg/mL; CBG, nmol/L × 0.058 = μg/mL; TC, nmol/L × 0.036 = μg/dL; FCI, nmol/nmol × 17.14 = nmol/mg; cFC, nmol/L × 0.036 = μg/dL).

The main findings of the present study were lower serum CBG levels in the AI group and a correlation between CBG and peak TC levels. These findings could explain the discordance in AI prevalence using the peak TC *vs*. peak FCI or peak cFC criteria. Thus, AI defined using the peak TC criterion in patients with BA could be overdiagnosed. This finding suggests that serum CBG was the main factor explaining the discordance between FC and TC in the diagnosis of AI, which is in agreement with the findings of a previous adult study (19).

Higher basal TC and a trend toward higher peak TC, despite lower serum CBG and higher basal FCI, peak FCI, basal cFC, and peak cFC, were observed in the higher PELD score group than in the lower PELD score group. These findings suggest that patients in the higher PELD score group might have more severe chronic systemic stress, and consequently, greater activation of the HPA axis. More severe impairment of cortisol clearance in patients with higher PELD scores may also explain this finding. Moreover, this study demonstrated that peak FCI and cFC, but not peak TC, were positively correlated with PELD scores. Lower serum CBG levels in the higher PELD score group partly contributed to the reduced peak TC. This finding is in agreement with that of adult patients (20). This might explain the absence of a correlation between peak TC and PELD scores. Taken together, these results suggest that the higher the severity of chronic liver disease, the higher the levels of both basal and stimulated biologically active free cortisol. However, studies in adults with stable cirrhosis have shown that AI prevalence has both a positive association and no association with the severity of liver disease (8). This discrepancy might be due to the different degrees of severity of liver diseases and the heterogeneity of the underlying etiology of liver disease. Additionally, differences in age-related responses to the ACTH test have been reported. Younger patients (< 5 yr old) have slightly higher peak TC and FC levels than older patients (32). This confounding factor may have affected the findings in the present study.

The median age of non-LT patients in this study was 4.1 (0.9–7.9) yr. In contrast to adult studies, the patients in this study were relatively young, and only patients with BA were included, indicating the homogeneity of the underlying etiology of liver disease. Disease severity, assessed using the PELD score, ranged from mild to moderate. The serum CBG and albumin levels were moderately low. Therefore, mildly severe liver disease in young children may primarily affect serum CBG levels and consequently reduce the measured serum TC levels. The direct detrimental effects of severe chronic liver disease in BA on adrenal function may not be demonstrated in this study.

The eight index patients with AI who met all three criteria had normal serum electrolyte levels and no episodes of hypoglycemia or adrenal crisis during the follow-up period, suggesting mild asymptomatic AI. They were advised to receive a stress dose of hydrocortisone only for moderate-to-severe illnesses. Annual adrenal function testing was performed. Regarding the type of AI, two patients had elevated plasma ACTH (presumptive diagnosis of primary AI). The remaining six patients had no elevated plasma ACTH levels (secondary AI). A previous study demonstrated that plasma ACTH levels were not significantly different between patients with cirrhosis with and without AI, suggesting that secondary rather than primary AI is more common (19). AI has also been proposed for the treatment of chronic liver disease. Impaired cholesterol synthesis may partially contribute to impaired cortisol production, leading to primary AI. Chronic hepatic inflammation (i.e., increased proinflammatory cytokines) may stimulate cortisol release and impair cortisol clearance in the liver, causing the HPA axis to become depressed. The mechanism underlying HPA axis dysfunction in chronic liver disease remains complex (33).

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Many previous studies have evaluated adrenal function in clinically stable adult cirrhotic patients and reported different prevalences of AI depending on the test used and different peak cortisol cutoff levels (7, 17, 22, 34–37). Assessment of adrenal function with the standard dose 250 µg ACTH test showed that the prevalence of AI was 39% by using peak TC < 500 nmol/L (< 18 µg/dL) and 12% by using peak FC < 33 nmol/L (< 1.2 µg/dL) (20). The other study reported that the prevalence of AI was 46% by using peak TC < 550 nmol/L (< 20 µg/dL) and 13% by using peak FCI < 0.7 nmol/nmol (< 12 nmol/mg) (22). These findings suggest that peak serum TC levels are likely to overdiagnose AI in clinically stable adult patients with cirrhosis. Furthermore, previous studies recommended using a 1 µg ACTH test rather than a 250 µg ACTH test for evaluating adrenal function because it is more physiological, sensitive, and appropriate for non-critically ill cirrhotic patients (8, 38). 250 µg ACTH test is preferable for adrenal function assessment in critically ill patients (8). Based on the Endocrine Society Clinical Practice Guideline for diagnosis of primary AI, a 250 µg ACTH test was recommended and a 1 µg ACTH test was acceptable (29). Currently, there is no recommended gold standard adrenal function test for patients with chronic liver diseases. Previous studies using the 1 µg ACTH test in adults and children with cirrhosis demonstrated that the respective AI prevalences were 38% (7) and 54% (10) defined by peak TC < 500 nmol/L (< 18 µg/dL) which are nearly comparable to the present study (35%). The strengths of this study are the unique chronic liver disease in children, BA, and the fact that the study population involved young children, whose data were still limited.

Study Limitations

We acknowledge the limitations of this study. Direct measurements of serum FC levels were not available. FCI and cFC, which are surrogate biomarkers of serum FC, were calculated from serum TC and CBG levels. These indirect serum FC estimates may not be identical to directly measured serum FC (31). Both FCI and cFC were calculated from serum TC and CBG levels, without considering serum albumin. Therefore, serum FC estimates should be interpreted with caution, particularly in patients with cirrhosis and low serum albumin (20). Further studies using direct measurements of serum FC and salivary cortisol and cortisone levels would better clarify our findings.

Conclusion

AI is common in patients with stable BA. Patients with BA frequently have relatively low serum CBG levels, which are associated with increased severity of BA. Reduced serum CBG levels reduce the measured peak serum TC level and consequently overdiagnose AI. Therefore, the diagnosis of AI based on peak serum TC levels needs to be cautiously interpreted. Peak FCI and peak cFC could help reduce the overdiagnosis of AI.

Conflicts of interests: The authors have nothing to declare.

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