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Serum Bilirubin and Albumin in Anti-*N*-Methyl-D-Aspartate Receptor Encephalitis

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Keywords
Bilirubin · Albumin · Antioxidant · Anti-*N*-methyl-D-aspartate receptor encephalitis · Autoimmune disorders

Abstract
Background and Objective: Low serum levels of bilirubin and albumin are associated with multiple autoimmune diseases, but their role in anti-*N*-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is unknown. ***Methods:*** Serum bilirubin and albumin levels were evaluated in 60 patients with anti-NMDAR encephalitis, 50 cryptococcal encephalitis, and 145 healthy controls (CTLs). Of the 60 anti-NMDAR encephalitis patients, 30 had a follow-up evaluation at 3 months after admission. ***Results:*** Serum bilirubin and albumin levels were both significantly lower in anti-NMDAR encephalitis than in CTLs, and serum bilirubin levels were significantly lower in anti-NMDAR encephalitis than in cryptococcal encephalitis. Serum bilirubin levels were significantly lower in patients with psychiatric symptoms, with severe impairment, and with limited responses to treatment than those without psychiatric symptoms, with mild impairment, and with favorable responses to treatment, respectively. A follow-up evaluation of 30 patients revealed that the modified Rankin Scale scores were significantly decreased after treatment. Serum bilirubin significantly associated with serum albumin, and plasma hemoglobin. ***Conclusions:*** Our results revealed for the first time an association between the serum levels of bilirubin in the anti-NMDAR encephalitis. Further studies investigating the role of bilirubin and albumin in anti-NMDAR encephalitis are required.

Introduction

Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is a severe neuropsychiatric disorder characterized by seizures, encephalopathy prominent memory, and behavioral deficits [1]. The disorder develops through the action of autoantibodies against the GluN1 subunit of the NMDAR [2]. The disease can occur in patients of all ages but is more often observed in young women and children [3]. Most patients respond to tumor removal and immunotherapies, including corticosteroids and in-

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travenous immunoglobulins (IVIG), or plasmapheresis and second-line agents, such as cyclophosphamide and rituximab [4].

Bilirubin is an end product of heme-containing proteins, such as hemoglobin, in aging red blood cells and is converted from biliverdin by biliverdin reductase [5]. Physiologically, bilirubin plays important roles due to its anti-oxidative and anti-inflammatory properties [6–10]. For example, bilirubin is an effective radical scavenger and inhibits the activity of nicotinamide adenine dinucleotide phosphate oxidase [11]. Albumin, which is mainly synthesized in the liver, has a higher total antioxidant capacity than other antioxidants, including bilirubin, α-tocopherol, ascorbic acid, and uric acid [12]. The antioxidant capacity of albumin, together with its property of modulating the intracellular signaling of neuronal or glial cells, contributes to its neuroprotective effect [13].

It has been shown that low levels of serum bilirubin and albumin are associated with a variety of autoimmune disorders, such as multiple sclerosis [14], neuromyelitis optica [15], myasthenia gravis [16], and systemic lupus erythematosus [17]. Very recently, Jang et al. [18] reported that serum albumin levels could predict the response to immune therapy in autoimmune encephalitis, suggesting an association between albumin and autoimmune encephalitis. However, the importance of bilirubin in anti-NMDAR encephalitis is still unknown. Here, we analyzed serum levels of bilirubin and albumin in anti-NMDAR encephalitis patients, and investigated the associations between serum bilirubin and albumin and various clinical parameters in these patients.

Methods

Patients and Controls

We recruited 60 patients with anti-NMDAR encephalitis and 50 with cryptococcal encephalitis, as well as 145 healthy controls (CTLs) for comparison. All patients had been hospitalized between July 2014 and June 2017 in the Third Affiliated Hospital of Sun Yat-Sen University. All the patients who entered into the study had encephalitis symptoms (including psychiatric symptoms, memory deficits, speech disturbances, seizures, movement disorders, loss of consciousness, sleep disorders, central hypoventilation), and some of them coincidentally had prodromal symptoms such as fever and/or headache. In this study, no subject showed hypertension, cardiopathy, diabetes, or hepatic/renal dysfunction. Anti-NMDAR encephalitis was defined according to diagnostic criteria [19]. Anti-NMDAR antibodies in the anti-NMDAR encephalitis patients were determined in cerebrospinal fluid (CSF) by cell-based assay using commercially available kits (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany). Cryptococcal encephalitis was defined as the isolation of cryptococcus neoformans in one or more CSF cultures, or positive CSF India ink and clinical features of encephalitis.

CSF examinations of patients were reviewed. Brain magnetic resonance imaging (MRI) was reviewed in the anti-NMDAR encephalitis. All anti-NMDAR encephalitis patients were screened with computed tomography, MRI, or B ultrasound at least once for systemic tumors. Treatments included tumor removal, the first-line immunotherapy including steroids, IVIG, alone or combined, and the second-line immunotherapy such as rituximab, azathioprine, and cyclophosphamide, alone or combined. The neurological status of each patient was assessed using the modified Rankin Scale (mRS) [20]. The initial treatment was recorded as a failure (limited response to treatment) if no sustained improvement occurred or if the mRS score remained at 4 or higher within 1 month of treatment.

Biochemical Assays

Venous blood was collected in the morning after an overnight fast when the patients arrived at our hospital. Levels of creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin (TBil), and albumin were determined in the blood using a Clinical Analyzer 7180-ISE (Hitachi High-Technologies, Tokyo, Japan). Levels of plasma hemoglobin were measured by an automatic hematology analyzer (XN2000; Sysmex, Japan).

Follow-Up Evaluations

We followed up 30 anti-NMDAR encephalitis patients at 3 months after treatment. Patients with anti-NMDAR encephalitis who were followed up received repeated assessments of mRS scores and serum TBil and albumin levels at our hospital.

Statistical Analysis

All statistical analyses were performed using SPSS 16.0 software (SPSS Inc, Chicago, IL, USA). All continuous variables were presented as the mean ± standard deviation if the data were normally distributed or as medians (min., max.) if the data were not normally distributed. To assess the significance of differences between groups, Student's *t* test was applied for the normally distributed data and Mann-Whitney U tests were performed when the data were not normally distributed. The χ^2 test was used for comparing the differences of gender between patients and healthy CTLs. Pearson's correlation and Spearman's rank correlation coefficient were used to evaluate correlations between different clinical parameters for normally distributed data and nonnormally distributed data, respectively. p values <0.05 were considered statistically significant.

Results

Demographic and Clinical Features of Patients and CTLs

As summarized in Table 1, the median time from onset until arrival in hospital was 30 days, and the median mRS, CSF WBC, and CSF total protein levels were 4.0, 5×10^6/L, and 0.26 g/L, respectively, in the anti-NMDAR en-

Table 1. Demographic features of patients and CTLs

	Anti-NMDAR encephalitis (n = 60)	Cryptococcal encephalitis (n = 50)	CTLs (n = 145)	p3	p2	p1
Age, years	28±15	33±11	31±19	0.129	0.538	0.284
Gender (male:female)	28:32	27:23	72:73	0.566	0.626	0.697
BMI	20.8 (10.4–29.1)	21.9 (10.8–30.2)	22.6 (15.1–30.8)	0.053	0.041	0.027
Time from onset until arrival in hospital, days	30 (2–1,800)	60 (7–2,160)	–	0.018	–	–
mRS	4.0 (1–5)	4.0 (0–5)	–	0.630	–	–
AST, U/L	25.4±13.3	21.7±9.6	22.9±8.0	0.069	0.389	0.176
ALT, U/L	24.8±14.2	24.9±11.7	21.6±13.0	0.561	0.076	0.127
Albumin, g/L	38.5±5.2	39.6.1±4.6	45.2±4.4	0.256	<0.001	<0.001
TBil, μmol/L	7.9±3.4	10.9± 3.7	12.3±4.1	<0.001	0.034	<0.001
CSF routine						
CSF WBC, $\times 10^6$/L	5.0 (0–197)	62.0 (2–454)	–	0.016	–	
CSF TP, g/L	0.26 (0.06–2.85)	0.81 (0.08–4.06)	–	0.027	–	
CSF Glu, mmol/L	3.31 (1.81–6.23)	1.92 (0.01–9.87)	–	0.291	–	
CSF CL, mmol/L	123.3 (107.4–132.2)	118.9 (103.4–139.5)	–	0.619	–	
Symptoms						
Headache	13(21.7)	50 (100)	–		–	
Fever	14 (23.3)	48 (85.7)			–	
Psychiatric symptoms	43 (71.7)	13 (26)	–		–	
Memory deficits	3 (5.0)	18 (36)	–		–	
Speech disturbances	21 (35.0)	8 (16)	–		–	
Seizures	23 (38.3)	20 (40)	–		–	
Movement disorders	28 (46.7)	27 (54)	–		–	
Loss of consciousness	12 (20.0)	19 (38)	–		–	
Sleep disorder	3 (5)	4 (8)	–		–	
Central hypoventilation	8 (13.3)	0	–		–	
Tumor comorbidity					–	
Ovarian teratoma	10 (16.7)	0	–			
Ovarian cysts	2 (3.3)	0	–			
Colon carcinoma	1 (1.7)	0	–			
Treatment						
Steroids	20 (33.3)	0	–			
Steroids plus IVIG	11 (18.3)	0	–			
Steroids plus RTX	9 (15)	0	–			
Steroids plus IVIG plus RTX	2 (3.3)	0	–			
Steroids plus CTX	2 (3.3)	0	–			
Steroids plus IVIG plus CTX	1 (1.7)	0	–			
Steroids plus AZA	3 (5)	0	–			
Steroids plus IVIG plus AZA	1 (1.7)	0	–			
Steroids plus tumor removal	2 (3.3)	0	–			
Steroids plus IVIG plus tumor removal	2 (3.3)	0	–			
Steroids plus RTX plus tumor removal	2 (3.3)	0	–			
Steroids plus IVIG plus RTX plus tumor removal	5 (8.3)	0	–			

Data are presented as the mean ± SD, median (range), or n (%). Anti-NMDAR, anti-*N*-methyl-D-aspartate receptor; CTLs, healthy controls; mRS, modified Rankin Scale; BMI, body mass index; CSF, cerebrospinal fluid; WBC, white blood cells; TP, total protein; Glu, glucose; CL, chlorine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBil, total bilirubin; IVIG, intravenous immunoglobulin; RTX, rituximab; CTX, cyclophosphamide; AZA, acetazolamide. p1, anti-NMDAR encephalitis versus CTLs; p2, cryptococcal encephalitis versus CTLs; p3, anti-NMDAR encephalitis versus cryptococcal encephalitis.

cephalitis patients. Of the 60 patients with anti-NMDAR encephalitis, 13 (21.7%) showed headache, 14 (23.3%) showed fever, 43 (71.7%) showed psychiatric symptoms, 3 (5%) showed memory deficits, 21 (35%) showed speech disturbances, 23 (38.3%) showed seizures, 28 (46.7%) showed movement disorders, 12 (20%) showed loss of consciousness, 3 (5 %) showed sleep disorders, and 8 (13.3 %) showed central hypoventilation.

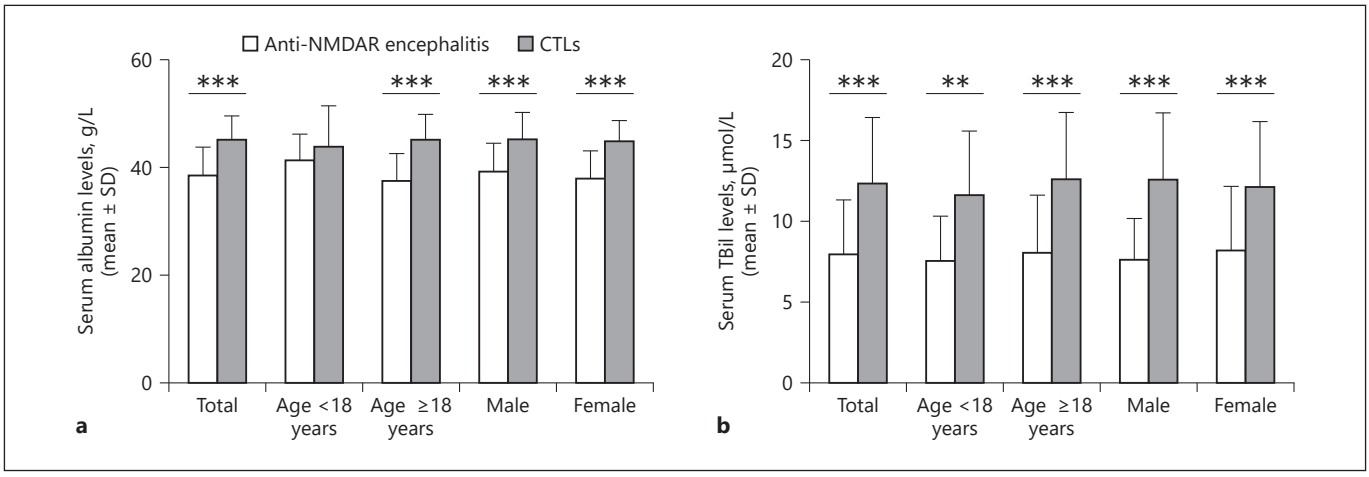

Fig. 1. a Comparison of albumin levels (**a**) and TBil levels (**b**) between anti-NMDAR encephalitis and CTLs (divided between all subjects and age <18 years, age ≥18 years, male and female subgroups). NMDAR, *N*-methyl-D-aspartate receptor; CTLs, healthy controls. *** $p < 0.001$, ** $p < 0.01$.

Comparison of Serum Albumin and TBil Levels among Anti-NMDAR Encephalitis, Cryptococcal Encephalitis, and CTLs

The concentrations of serum TBil in patients with anti-NMDAR encephalitis were significantly lower than those in cryptococcal encephalitis ($p < 0.001$) and CTLs ($p < 0.001$), and serum TBil concentrations in cryptococcal encephalitis patients were also significantly lower than CTLs ($p < 0.05$; Table 1). Serum albumin levels were significantly lower in anti-NMDAR encephalitis and cryptococcal encephalitis than in CTLs ($p < 0.001$, $p < 0.001$, respectively). Although serum albumin levels were lower in anti-NMDAR encephalitis than in cryptococcal encephalitis, the difference was not significant (Table 1).

Analysis according to gender demonstrated that serum albumin and TBil levels in males and females were both significantly lower in patients with anti-NMDAR encephalitis than in CTLs, respectively. (Fig. 1a, b). According to age, serum albumin and TBil levels were significantly lower in patients aged ≥18 years than in CTLs (Fig. 1a, b). Serum TBil levels were also significantly lower in patients aged <18 years than in CTLs (Fig. 1b), while the difference of serum albumin between the subgroup with age <18 years was not statistically significant (Fig. 1a).

Comparison of Serum Albumin and TBil Levels between Subgroups of Patients with Anti-NMDAR Encephalitis

We then investigated the association of serum albumin and TBil with subgroups of patients with anti-NMDAR encephalitis. Patients with anti-NMDAR encephalitis were divided into subgroups according to age, gender, mRS, symptom, brain MRI, BMI, presence of tumor, and response to therapy. As summarized in Table 2, patients aged ≥18 years had significantly lower serum albumin levels than those aged <18 years ($p = 0.014$), patients with mRS ≥4 had significantly lower serum TBil levels than those with mRS <4 ($p < 0.001$), patients with psychiatric symptoms had significantly lower serum bilirubin levels than those without psychiatric symptoms ($p = 0.015$), and patients with limited responses to treatment had significantly lower serum TBil levels than those with favorable treatment outcomes ($p = 0.034$). No other significant association was identified (Table 2).

Follow-Up Evaluation of Serum Albumin and TBil Levels in Anti-NMDAR Encephalitis Patients following Treatment

In order to decrease the influence of the immunotherapy, we divided anti-NMDAR encephalitis patients into two subgroups (first-line immunotherapy group, and first-line combined with second-line immunotherapy group) according to the treatments received. As shown in Table 3, after treatment, only mRS scores were significantly decreased in both of the subgroups. Although serum levels of albumin and TBil were all increased after treatment in the two subgroups, they were not significant. Also, changes in mRS scores were not correlated with changes in TBil or albumin levels in either of the subgroups (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000494801).

Table 2. Serum albumin and TBil levels in patients with anti-NMDAR encephalitis

Variables	Albumin, g/L		TBil, μmol/L	
	median (min.–max.)	*p* value	median (min.–max.)	*p* value
Age				
<18 years (*n* = 15)	43 (28–49)		7.0 (4.7–14.9)	
≥18 years (*n* = 45)	36.3 (27.2–45.6)	0.014	7.5 (3.3–23.8)	0.657
Gender				
Male (*n* = 28)	40.5 (27.2–45.6)		7.3 (3.3–15.3)	
Female (*n* = 32)	37.2 (28–49)	0.325	7.01 (3.9–23.8)	0.511
mRS				
<4 (*n* = 26)	39.0 (27.2–49.0)		9.3 (4.8–23.8)	
≥4 (*n* = 34)	37.45 (28.0–45.2)	0.177	6.45 (3.3–12.8)	<0.001
BMI				
<20 (*n* = 26)	40.5 (32–49)		7.06 (3.9–13.9)	
≥20 (*n* = 34)	36.3 (27.2–45.6)	0.084	7.10 (3.3–23.8)	0.541
Prodromal symptom				
With (*n* = 21)	37.8 (28.0–45.6)		6.9 (3.3–15.3)	
Without (*n* =39)	40.3 (27.2–49.0)	0.316	7.6 (3.9–23.8)	0.443
Psychiatric symptoms				
With (*n* = 43)	37.2 (27.2–49.0)		6.9 (3.3–14.9)	
Without (*n* = 17)	41.9 (28.0–45.6)	0.129	8.4 (5.6–23.8)	0.015
Brain MRI				
Normal (*n* = 25)	37.8 (27.2–49)		6.7 (4.1–15.3)	
Abnormal (*n* = 35)	38.5 (28–45.6)	0.810	7.5 (3.3–23.8)	0.242
Tumor				
With (*n* = 13)	38.5 (31.4–44.6)		6.64 (4.1–12.8)	
Without (*n* = 47)	38.3 (27.2–49)	0.781	7.2 (3.3–23.8)	0.306
Response to therapy				
Favorable (*n* = 45)	39.7 (27.2–49.0)		7.6 (3.3–23.8)	
Limited (*n* = 15)	34.6 (28.0–44.6)	0.061	6.4 (4.1–12.2)	0.034

Table 3. Demographics of the 30 anti-NMDAR encephalitis patients with 3 months of follow-up

	First-line immunotherapy (*n* = 17)			First-line combined with second-line immunotherapy (*n* = 13)		
	before treatment	after treatment	*p* value	before treatment	after treatment	*p* value
Sex (male:female)	7:10	7:10	–	6: 7	6:7	–
Age, years	26 (12–53)	26 (12–53)	–	25 (12–65)	26.0 (12–65)	–
BMI	19.3 (16.1–26.0)	21.9 (16.7–29.0)	0.053	21.4 (14.6–29.1)	22.1 (15.8–29.7)	0.082
Time from onset until arrival in hospital, days	30 (5–120)	120 (95–210)	–	60 (7–1,800)	150 (97–1,890)	–
mRS	4 (3–5)	2 (0–5)	0.003	4 (3–5)	2 (0–5)	0.002
Albumin, g/L	38.4±5.1	40.3±3.7	0.234	37.8±4.8	40.4±4.6	0.174
TBil, μmol/L	7.0 (3.9–9.5)	7.9 (3.8–22.5)	0.263	6.6 (4.1–9.7)	8.2 (2.6–15.9)	0.212

Values are presented as *n*, the median (range), or mean ± SD. Anti-NMDAR, anti-*N*-methyl-D-aspartate receptor; mRS, modified Rankin Scale; TBil, total bilirubin.

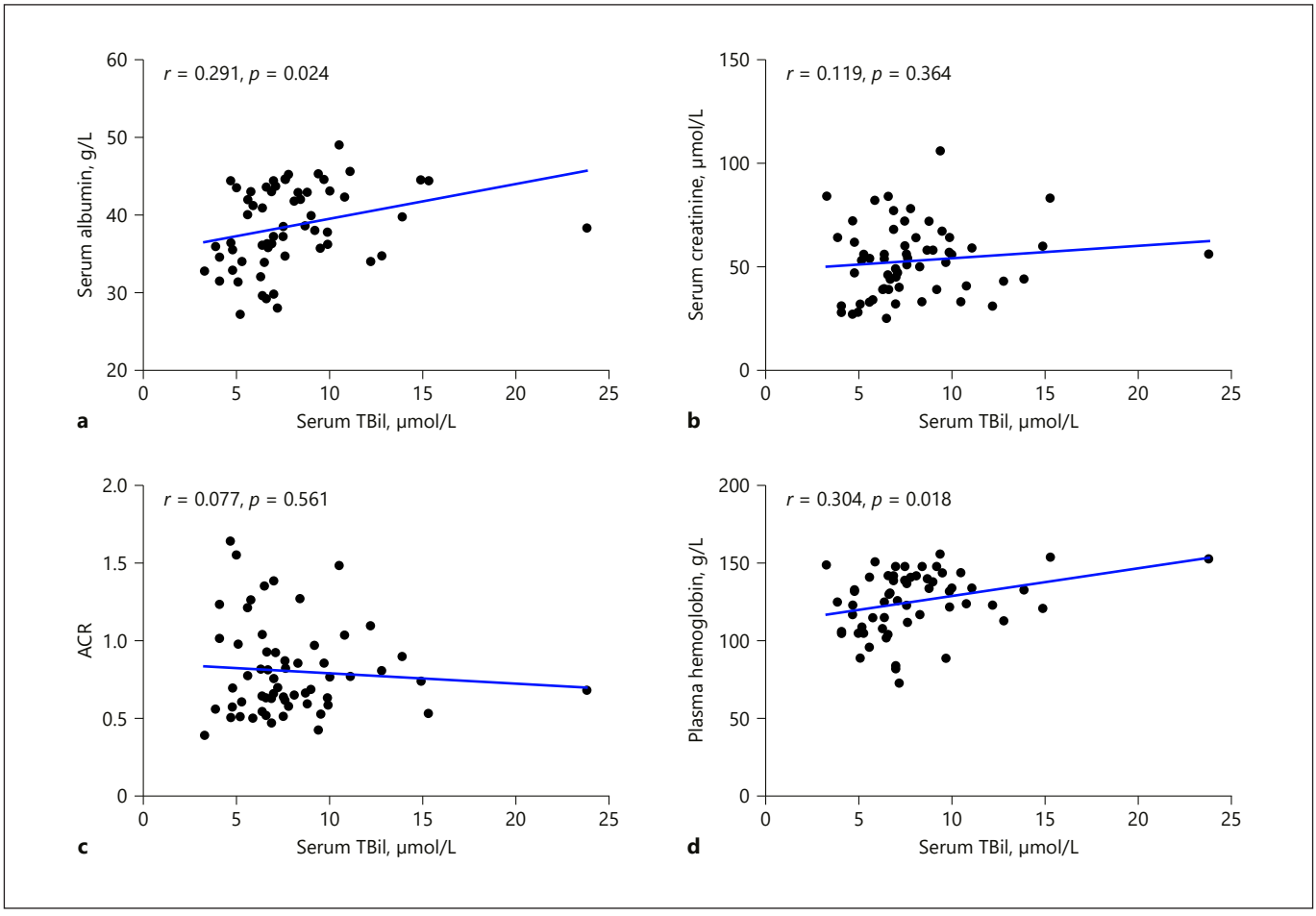

Fig. 2. The relationship between serum TBil and albumin (**a**), creatinine (**b**), ACR (**c**), and hemoglobin (**d**) in anti-NMDAR encephalitis patients (n = 60). ACR, albumin to creatinine ratio.

Associations among Serum TBil Levels and Serum Albumin, Creatinine, Albumin to Creatinine Ratio, Hemoglobin, and Clinical Characteristics in Anti-NMDAR Encephalitis Patients

We next investigated relationships among serum TBil levels and serum albumin, creatinine, albumin to creatinine ratio (ACR), and hemoglobin in patients with anti-NMDAR encephalitis. As shown in Figure 2, serum levels of TBil were significantly correlated with serum levels of albumin (r = 0.291, p = 0.024) and hemoglobin (r = 0.304, p = 0.018). Although serum TBil levels were positively associated with creatinine levels (r = 0.119, p = 0.364) and inversely associated with ACR (r = –0.077, p = 0.561), these correlations were not significant.

The relationships between serum albumin and TBil levels and clinical characteristics and CSF parameters in patients with anti-NMDAR encephalitis were also evaluated (Table 4). Significant correlations were identified between albumin levels and age (r = –0.399, p = 0.002), and between TBil levels and mRS (r = –0.540, p < 0.001). However, correlations between serum albumin or TBil levels and gender, BMI, time from onset until arrival in hospital, and CSF parameters (WBC, total protein) were not significant. To eliminate the influence of gender, we performed stratified analysis by dividing patients into male and female groups (Table 4). In male patients, there were significant correlations between serum levels of TBil and age (r = 0.392, p = 0.039) and mRS (r = –0.432, p = 0.022). Meanwhile, in female patients, there were significant correlations between albumin levels and age (r = –0.592, p < 0.001) and mRS (r = –0.356, p = 0.046), and between TBil levels and mRS (r = –0.613, p < 0.001).

Table 4. Correlation coefficients generated between serum albumin and TBil and clinical characteristics and CSF parameters in patients with anti-NMDAR encephalitis

Variable	Total				Male				Female			
	Albumin		TBil		Albumin		TBil		Albumin		TBil	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Gender	0.176	0.177	−0.005	0.971	–	–	–	–	–	–	–	–
Age	−0.399**	0.002	0.154	0.241	−0.272	0.161	0.392*	0.039	−0.607**	<0.001	−0.026	0.887
mRS	−0.167	0.203	−0.540**	<0.001	0.067	0.734	−0.432*	0.022	−0.356	0.046*	−0.613**	<0.001
BMI	−0.145	0.270	−0.009	0.945	−0.301	0.119	−0.160	0.417	−0.112	0.542	−0.027	0.883
Time from onset until arrival in hospital	−0.025	0851	−0.012	0.927	−0.034	0.862	−0.018	0.928	−0.012	0.947	0.01	0.956
CSF WBC	−0.119	0.373	−0.181	0.175	0.178	0.364	−0.192	0.328	−0.344	0.062	−0.151	0.427
CSF TP	0.171	0.201	0.148	0.266	0.339	0.078	0.212	0.279	−0.016	0.933	0.180	0.342

*$p < 0.05$, **$p < 0.01$. Pearson's correlations were used to evaluate the correlations between serum albumin and TBil and age. Spearman's correlations were used to evaluate the correlations between serum albumin and TBil and gender, mRS, BMI, disease duration, CSF WBC, and CSF TP. For all abbreviation definitions, see footnotes to Table 1.

Discussion

Our results showed that patients with anti-NMDAR encephalitis had lower levels of bilirubin and albumin than cryptococcal encephalitis patients and healthy subjects. Serum bilirubin levels were negatively correlated with disease severity and positively correlated with serum albumin and plasma hemoglobin levels. In the follow-up evaluation, severity scores were significantly decreased in patients after treatment. This study revealed for the first time an association between the serum level of bilirubin and anti-NMDAR encephalitis. In addition, it also confirmed the association between serum levels of albumin and anti-NMDAR encephalitis.

Accumulating evidence suggests that bilirubin might play a role in the parthenogenesis of autoimmune diseases. For example, it has been reported that treatment with bilirubin effectively suppresses experimental autoimmune encephalomyelitis (EAE) while depletion of endogenous bilirubin dramatically exacerbates EAE [21], suggesting a protective role of bilirubin in the development of EAE. Furthermore, bilirubin could protect against EAE by inhibiting $CD4^+$ T cell reactivity through the inhibition of costimulator activities, suppression of immune transcription factor activation, and downregulation of inducible MHC class II expression [21]. Besides EAE, experimental colitis has also been shown to be regulated by bilirubin in a recent study in which Longhi et al. [22] found that bilirubin suppressed immune responses of Th17 cells by upregulating CD139. In addition, Trujillo-Ochoa et al. [23] demonstrated that bilirubin might exhibit an anti-inflammatory role by regulating regulatory T cell activity via the T cell immunoglobulin domain and mucin domain 3 (TIM-3) during acute hepatitis A virus infection. Thus, it is conceivable that bilirubin might contribute to the development of anti-NMDAR encephalitis by regulating autoreactive T cells or regulatory cells.

In the follow-up evaluation, severity scores were significantly decreased, and serum bilirubin and albumin levels were both increased after treatment, although they were not significant. Certainly, serum levels of bilirubin and albumin might be changed with other factors, such as treatment, nutritional health, disease severity, and time in hospital. Recently, Jang et al. [18] showed that changes occurred in albumin levels with treatment (IVIG) and different albumin levels predicted different responses to immune therapy in autoimmune encephalitis. In addition, the patients could get enough nutrition when they were recovering. Therefore, more research is needed to reveal the relationship between serum bilirubin and albumin and disease prognosis.

Psychiatric symptoms are the main clinical feature of anti-NMDAR encephalitis [24]. Several studies have suggested that psychological stress induces the production of reactive-oxygen species and psychological stress provokes bilirubin oxidation in vivo [25, 26]. For example, Miyaoka et al. [26] found that the concentration of bilirubin oxidative metabolites (biopyrins) was increased in urine from patients with psychiatric disorders, suggesting that psychotic states associated with an increase in the oxidative metabolites of bilirubin. In our study, 71.7% (43/60) of patients with anti-NMDAR encephalitis had

psychiatric symptoms, and patients with psychiatric symptoms had significantly lower serum bilirubin levels than CTLs, supporting the notion.

It has been reported that serum bilirubin concentrations are positively correlated with hemoglobin concentration and inversely correlated with ACR among Indigenous Australians [27]. In the current study, we also found that serum bilirubin levels were significantly positively correlated with hemoglobin and albumin levels, and insignificantly inversely correlated with ACR in anti-NMDAR encephalitis. In humans, a lower serum albumin concentration has also been found in the elderly [28]. In rats, the serum albumin concentration increases from 3 to 7 months then decreases from 12 to 20 months of age [29]. In this study, we found that serum albumin levels were significantly inversely associated with age, and older anti-NMDAR encephalitis patients had significantly lower serum albumin levels compared with younger patients. It may be associated with insufficient nutrients absorbed or more oxidative stress suffered in the elderly.

There were several limitations to our study that should not be ignored. First, the number of studied subjects in the current study was relatively small, and CTLs were not well matched regarding age, sex, and BMI. Second, it has been suggested that cytochrome P450 2A5 (CYP2A5) plays a key role in bilirubin clearance [30]; however, P450 2A5 genotypes and their activity were not considered in this study. Third, we did not determine the total antioxidant capacity of bilirubin and albumin in anti-NMDAR encephalitis. Fourth, this is only a preliminary and descriptive study, which lacks evidence for biological and pathological mechanisms. Last but not least, the prodromal and encephalic phases of the disease were not analyzed separately in the current study, as the treatment and the response to the treatment might influence the levels of serum bilirubin and albumin.

The current study has demonstrated that levels of serum bilirubin and albumin are reduced in anti-NMDAR encephalitis patients, and levels of serum bilirubin are associated with disease activity, serum albumin, and plasma hemoglobin. This finding suggests that bilirubin and albumin might associate with the development of anti-NMDAR encephalitis. Thus, future studies exploring the role of bilirubin and albumin in the disease are required.

Acknowledgments

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Statement of Ethics

This research was approved by the ethics committee of the Third Affiliated Hospital of Sun Yat-Sen University. Informed written consent was obtained from all the patients or their representatives.

Disclosure Statement

The authors declare that they have no competing interests.

References

1 Vincent A, Bien CG. Anti-NMDA-receptor encephalitis: a cause of psychiatric, seizure, and movement disorders in young adults. Lancet Neurol. 2008 Dec;7(12):1074–5.
2 Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci. 2010 Apr;30(17):5866–75.
3 Florance NR, Davis RL, Lam C, Szperka C, Zhou L, Ahmad S, et al. Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in children and adolescents. Ann Neurol. 2009 Jul;66(1):11–8.
4 Titulaer MJ, McCracken L, Gabilondo I, Armangué T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet Neurol. 2013 Feb;12(2):157–65.
5 Lin JP, Vitek L, Schwertner HA. Serum bilirubin and genes controlling bilirubin concentrations as biomarkers for cardiovascular disease. Clin Chem. 2010 Oct;56(10):1535–43.
6 Matsuoka Y, Masuda H, Yokoyama M, Kihara K. Protective effects of bilirubin against cyclophosphamide induced hemorrhagic cystitis in rats. J Urol. 2008 Mar;179(3):1160–6.
7 Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science. 1987 Feb;235(4792):1043–6.
8 Wang WW, Smith DL, Zucker SD. Bilirubin inhibits iNOS expression and NO production in response to endotoxin in rats. Hepatology. 2004 Aug;40(2):424–33.
9 Wu TW, Carey D, Wu J, Sugiyama H. The cytoprotective effects of bilirubin and biliverdin on rat hepatocytes and human erythrocytes and the impact of albumin. Biochem Cell Biol. 1991 Dec;69(12):828–34.
10 Qaisiya M, Coda Zabetta CD, Bellarosa C, Tiribelli C. Bilirubin mediated oxidative stress involves antioxidant response activation via Nrf2 pathway. Cell Signal. 2014 Mar;26(3):512–20.
11 Lanone S, Bloc S, Foresti R, Almolki A, Taillé C, Callebert J, et al. Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implications for protection against endotoxic shock in rats. FASEB J. 2005 Nov;19(13):1890–2.

12 Ihara H, Hashizume N, Hasegawa T, Yoshida M. Antioxidant capacities of ascorbic acid, uric acid, alpha-tocopherol, and bilirubin can be measured in the presence of another antioxidant, serum albumin. J Clin Lab Anal. 2004;18(1):45–9.
13 Prajapati KD, Sharma SS, Roy N. Current perspectives on potential role of albumin in neuroprotection. Rev Neurosci. 2011;22(3):355–63.
14 Ljubisavljevic S, Stojanovic I, Vojinovic S, Milojkovic M, Dunjic O, Stojanov D, et al. Association of serum bilirubin and uric acid levels changes during neuroinflammation in patients with initial and relapsed demyelination attacks. Metab Brain Dis. 2013 Dec;28(4):629–38.
15 Peng F, Yang Y, Liu J, Jiang Y, Zhu C, Deng X, et al. Low antioxidant status of serum uric acid, bilirubin and albumin in patients with neuromyelitis optica. Eur J Neurol. 2012 Feb;19(2):277–83.
16 Yang D, Su Z, Wu S, Bi Y, Li X, Li J, et al. Low antioxidant status of serum bilirubin, uric acid, albumin and creatinine in patients with myasthenia gravis. Int J Neurosci. 2016 Dec;126(12):1120–6.
17 Vítek L, Muchová L, Jančová E, Pešičková S, Tegzová D, Peterová V, et al. Association of systemic lupus erythematosus with low serum bilirubin levels. Scand J Rheumatol. 2010 Nov;39(6):480–4.
18 Jang Y, Lee ST, Kim TJ, Jun JS, Moon J, Jung KH, et al. High albumin level is a predictor of favorable response to immunotherapy in autoimmune encephalitis. Sci Rep. 2018 Jan;8(1):1012.
19 Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol. 2016 Apr;15(4):391–404.
20 van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke. 1988 May;19(5):604–7.
21 Liu Y, Li P, Lu J, Xiong W, Oger J, Tetzlaff W, et al. Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. J Immunol. 2008 Aug;181(3):1887–97.
22 Longhi MS, Vuerich M, Kalbasi A, Kenison JE, Yeste A, Csizmadia E, et al. Bilirubin suppresses Th17 immunity in colitis by upregulating CD39. JCI Insight. 2017 May;2(9):2.
23 Trujillo-Ochoa JL, Corral-Jara KF, Charles-Nino CL, Panduro A, Fierro NA. Conjugated bilirubin upregulates TIM-3 expression on CD4+CD25+ T Cells: anti-inflammatory implications for hepatitis A virus infection. Viral Immunol. 2018 Apr;31(3):223–32.
24 Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol. 2008 Dec;7(12):1091–8.
25 Yamaguchi T, Shioji I, Sugimoto A, Yamaoka M. Psychological stress increases bilirubin metabolites in human urine. Biochem Biophys Res Commun. 2002 Apr;293(1):517–20.
26 Miyaoka T, Yasukawa R, Yasuda H, Shimizu M, Mizuno S, Sukegawa T, et al. Urinary excretion of biopyrrins, oxidative metabolites of bilirubin, increases in patients with psychiatric disorders. Eur Neuropsychopharmacol. 2005 May;15(3):249–52.
27 Hughes JT, Barzi F, Hoy WE, Jones GR, Rathnayake G, Majoni SW, et al. Bilirubin concentration is positively associated with haemoglobin concentration and inversely associated with albumin to creatinine ratio among Indigenous Australians: eGFR Study. Clin Biochem. 2017 Dec;50(18):1040–7.
28 Gom I, Fukushima H, Shiraki M, Miwa Y, Ando T, Takai K, et al. Relationship between serum albumin level and aging in community-dwelling self-supported elderly population. J Nutr Sci Vitaminol (Tokyo). 2007 Feb;53(1):37–42.
29 Barber BJ, Babbitt RA, Parameswaran S, Dutta S. Age-related changes in rat interstitial matrix hydration and serum proteins. J Gerontol A Biol Sci Med Sci. 1995 Sep;50(5):B282–7.
30 Kim SD, Antenos M, Squires EJ, Kirby GM. Cytochrome P450 2A5 and bilirubin: mechanisms of gene regulation and cytoprotection. Toxicol Appl Pharmacol. 2013 Jul;270(2):129–38.