ASSESSMENT OF ADRENOCORTICAL DYSFUNCTION IN PATIENTS WITH STABLE LIVER CIRRHOSIS

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Abstract

Introduction. Relative adrenal insufficiency (RAI) is common in the setting of critical illness as well as in hemodynamically unstable cirrhotic patients with sepsis. Several studies have also shown that RAI is frequent in patients with stable cirrhosis without sepsis. The aim of this study was to prospectively assess the incidence of RAI in patients with stable cirrhosis.

Patients and Methods. Forty-seven patients with hemodynamically stable liver cirrhosis without sepsis were prospectively included. RAI, assessed by using low dose-short Synacthen test (LD-SST), was defined as either a basal total cortisol concentration below 3.6 µg/dL or a peak total serum cortisol ≤ 16 µg/dL at 30 min after stimulation.

Results. RAI was present in 10 (21.3%) of 47 cirrhotic patients. Peak cortisol level was negatively correlated with the severity of cirrhosis evaluated by Child-Turcotte-Pugh (CTP) ($r=-0.46$; $P=0.001$) and Model for End-Stage Liver Disease (MELD) ($r=-0.51$; $P=0.001$) scores. The frequency of RAI increased from CTP-A (10%) to CTP-B (30%) to CTP-C (60%).

Conclusion. RAI diagnosed by LD-SST is frequent in patients with stable cirrhosis and is related to the severity of liver disease. Further studies are needed to define clinical importance of RAI in stable cirrhotic patients.

Key words: Relative adrenal insufficiency, Liver cirrhosis, Hepato-adrenal syndrome, Cortisol.

INTRODUCTION

Liver cirrhosis is a major cause of mortality worldwide, often with severe sepsis as the terminal event. Patients with cirrhosis have an increased risk to develop bacterial infections, severe sepsis, and septic shock, complications which are associated with high mortality rate (1-3). Several studies have reported that in septic patients the adrenal glands respond inappropriately to stimulation, and that the treatment with corticosteroids may decrease mortality

in such patients (4-6). Both cirrhosis and septic shock share many hemodynamic abnormalities such as hyperdynamic circulatory failure, increased cardiac output, decreased peripheral vascular resistance, and hypo-responsiveness to vasopressors; consequently, it has been reported that adrenal insufficiency is common in critically ill cirrhotic patients (7, 8). Adrenal insufficiency is defined by a decreased production or action of glucocorticoids due to a structural damage of adrenal glands (“primary adrenal insufficiency”) or an impairment of the hypothalamic-pituitary axis (“secondary adrenal insufficiency”). In addition to critically ill cirrhotic patients, adrenal insufficiency has also been reported in stable and decompensated cirrhosis without sepsis (9-11) or in early and late post-liver transplantation (12, 13). The term “hepato-adrenal syndrome” was first used by Marik *et al.* (8) to define adrenal insufficiency in critically ill cirrhotic patients, and it suggests that adrenocortical dysfunction may be a feature of liver disease *per se* with a different pathogenesis from that occurring in septic shock. Recently, two terms have been used to define the adrenocortical insufficiency, namely “critical illness related corticosteroid insufficiency” (CIRCI) defined as “inadequate cellular corticosteroid activity for the severity of the patient’s illness” (14), and “Relative adrenal insufficiency” (RAI), the term given to inadequate production of cortisol with respect to the severity of illness (15).

The mechanisms of RAI in cirrhotic patients are not completely understood, although impaired synthesis in total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, as well as increased levels of proinflammatory cytokines have been suggested (7, 14, 16). Coagulopathy, frequent in liver cirrhosis, could produce adrenal hemorrhage and infarction and thus may lead to primary adrenal dysfunction (8). The

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adrenal glands do not store cortisol, but synthesize it during stress from the main precursor, HDL cholesterol. Thus, cortisol synthesis may be impaired as HDL cholesterol levels are frequently low in the setting of liver cirrhosis (8, 16). Systemic inflammation, leading to corticosteroid resistance (14) may also have a role in the development of RAI in cirrhotic patients. Increased levels of circulating endotoxin (lipopolysaccharide) and TNF- α inhibit cortisol synthesis, limiting the delivery of HDL cholesterol to the adrenal gland (14, 16).

The prevalence of RAI in cirrhotic patients varies widely according to the stage of liver disease: 10%-92% in critically ill patients (8, 17, 18), 9%-60% in compensated or stable cirrhosis (11, 19, 20), and 30%-39% in those with decompensated cirrhosis (variceal bleeding, ascites) (10, 21). In addition, the prevalence varies with the diagnostic criteria defining RAI and the methodology used. In the absence of a gold standard test, standard dose-short Synacthen test (SD-SST) remains the most used test to evaluate the adrenal function in critically ill cirrhotics, while low dose-short Synacthen test (LD-SST) seems to be more appropriate in those with stable cirrhosis (22).

The aim of our study was to prospectively assess the incidence of RAI in patients with hemodynamically stable liver cirrhosis without sepsis, using LD-SST.

PATIENTS AND METHODS

Patients

This prospective observational study includes patients over 18 years of age with stable and without sepsis liver cirrhosis admitted to the Institute of Gastroenterology and Hepatology, an academic tertiary referral center for adults with liver disease in the North-Eastern Romania, between February 2015 and July 2015. Cirrhosis was diagnosed by liver biopsy or liver stiffness measurement (Fibroscan) in addition to typical clinical, laboratory and ultrasound findings. All patients were hemodynamically stable and without signs of sepsis, hospitalized for a check-up of their cirrhosis. Exclusion criteria were: history of hypothalamic-pituitary disease, glucocorticoid treatment within the preceding 6 months, active bacterial infection or recent history of infection, major organ failure, hepatocellular carcinoma, pregnancy, HIV infection, hemodynamic instability (mean arterial pressure < 60 mmHg or vasopressor dependency).

A clinical evaluation was performed during the first day of hospitalization, as well as a chest X-ray, ECG,

standard liver and kidney function tests, measurement of serum total cholesterol, HDL- and LDL- cholesterol. The severity of the liver disease was assessed by the Model for End-Stage Liver Disease (MELD) and Child-Turcotte-Pugh (CTP) scores (23).

Adrenal function assessment

Adrenal function was assessed by using the LD-SST. Blood samples were obtained in the morning after an overnight fast, immediately before and 30 minutes after the intravenous injection of 1 μ g of synthetic adrenocorticotrophic hormone (Synacthen Sigma-tau industrie farmaceutice riunite S.P.A, Pomezia, Italy). The solution used for adrenal stimulation was prepared by adding 250 μ g Synacthen into a 250 mL saline recipient; thus, 1 mL of this solution contains 1 μ g Synacthen (24). All LD-SSTs were performed by a single author (SC). Normal response to LD-SST at 30 minutes after Synacthen was defined as a plasma cortisol concentration $\geq 16\mu$ g/dL.

Total cortisol concentrations were measured using chemiluminescence immunoassay on an Immulite 2000 analyzer (Siemens Medical Diagnostics Solutions, Erlangen, Germany).

RAI was diagnosed using either a basal total cortisol concentration below 3.6 μ g/dL (25) or a peak total cortisol <16 μ g/dL (26). The peak cortisol concentration was defined as the highest cortisol value after LD-SST.

All patients gave written informed consent prior to the enrollment, and the study was approved by local ethics committee.

Statistical analysis

Data was processed using SPSS statistical package version 10.0. Categorical variables were expressed as absolute values and percentages and compared using the χ^2 test or the Fisher's exact test (for small samples). Continuous variables were expressed as mean $\pm$ standard deviation or as median and the quartiles (Q1-Q3) as appropriate. The independent samples t-test or the Mann-Whitney test were used accordingly for continuous variables with normal and non-normal distribution, respectively. Correlations were evaluated using Pearson's correlation coefficient. Statistical significance was considered for a value of $P < 0.05$.

RESULTS

Demographic and laboratory data of cirrhotic patients with and without RAI are presented in Table 1.

Table 1. Demographic and laboratory characteristics of cirrhotic patients with and without relative adrenal insufficiency

Characteristics	Patients with RAI (n=10)	Patients without RAI (n=37)	P value
Age (years)	60.5 ± 9.2	61.3 ± 8.9	0.799
Gender (male:female)	7:3	21:16	0.189
Etiology			
Viral (C,B)	3 (30%)	9 (24.3%)	0.866
Alcohol	6 (60%)	25 (67.6%)	
Other	1 (10%)	3 (8.1%)	
CTP score	9.5 ± 2.6	8.78 ± 2.4	0.02
CTP-A	1 (10%)	13 (35.1%)	0.157
CTP-B	3 (30%)	14 (37.8%)	
CTP-C	6 (60%)	10 (27%)	
MELD score	20 (14-23.5)	11 (9-14)	0.001
ALT (UI/L)	31.5 (19.7-70)	42 (24-58)	0.76
AST (UI/L)	61 (30.5-98)	42.5 (32-81)	0.37
Albumin (g/dL)	2.3 ± 0.6	3.3 ± 0.7	<0.001
Total bilirubin (mg/dL)	2.5 (1.12-33)	1.7 (0.6-19.7)	0.02
Prothrombine activity (%)	50.9 ± 11.1	74.1 ± 17.0	<0.001
INR	1.5 ± 0.1	1.3 ± 0.2	0.001
Leucocytes (X 10 ⁹ /L)	3.4 (2.7-6.7)	4.1 (3.6-4.8)	0.469
Trombocytes (X 10 ⁹ /L)	95.7 ± 49.0	121.8 ± 40.5	0.092
Na (mmol/L)	130.8 ± 6.9	135.8 ± 6.6	0.06
Creatinine (mg/dL)	1.41 ± 0.6	1.0 ± 0.3	0.68
LDL – cholesterol (mg/dL)	68.5 ± 8.7	94.2 ± 22.8	<0.001
HDL – cholesterol (mg/dL)	23.2 ± 4.17	34.8 ± 15.8	<0.001

Abbreviations: RAI relative adrenal insufficiency, HDL high density lipoprotein, LDL low density lipoprotein, INR international normalized ratio, MELD Model for End-Stage Liver Disease, CTP Child-Turcotte-Pugh, ALT alanine aminotransferase, AST aspartate aminotransferase

Values are presented as numbers and percentages, mean ± SD, median (IQR). P-value < 0.05 is considered statistically significant.

Table 2. Baseline and post low dose-short synacthen test results

	RAI present (n=10)	RAI absent (n=37)	P-value
Baseline total cortisol (µg/dL)	8.2 (4-9.8)	13.8 (9.8-16)	0.001
Post LD-SST			
Peak total cortisol (µg/dL)	12.1 ± 2.2	21.6 ± 5.1	< 0.001

Abbreviations: RAI relative adrenal insufficiency, LD-SST low dose-short synacthen test

Values are presented as mean ± SD and median (IQR). P-value < 0.05 is considered statistically significant.

A total of 47 patients (28 men and 19 women, age 61.1 ± 8.9 years) with cirrhosis were included in this study. Out of these, 10 (21.3%) patients were diagnosed with RAI defined as a peak total cortisol level less than 16 µg/dL at 30 minutes after stimulation. There were no significant differences between patients with RAI and those without RAI regarding mean age, gender, etiology of cirrhosis, most of laboratory indices such as serum creatinine, sodium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), leucocytes and thrombocytes. However, there were significant differences between the values of the CTP and MELD scores, international normalized ratio (INR), HDL-and LDL-cholesterol levels, albumin and bilirubin.

The results of the LD-SST are presented

in Table 2. Baseline levels of total cortisol were statistically different between cirrhotic patients with and those without RAI [8.2 (4-9.8) µg/dL vs. 13.8 (9.8-16) µg/dL; P=0.001]. None of the patients had at baseline a total cortisol < 3.6 µg/dL to fulfil the criteria for RAI. The peak total cortisol was inversely related to the severity of liver disease based on CTP and MELD scores (r = -0.46, P=0.001 and r=-0.51, P<0.001, respectively). The mean CTP and MELD scores were significantly higher in the group of patients with RAI than in those without RAI [9.5 ± 2.6 vs. 8.78 ± 2.4, P=0.02 and 20 (14-23.5) vs. 11 (9-14), P=0.001, respectively]. In addition, the frequency of RAI varied progressively from 10%, in CTP-A patients to 60%, in CTP-C class patients.

DISCUSSION

Our study found that RAI, defined by a serum total cortisol concentration $< 16 \mu\text{g/dL}$ after stimulation with $1 \mu\text{g}$ Synacthen, was present in 21.3% of patients with hemodynamically stable liver cirrhosis without sepsis. This result is in accordance with those reported by other studies (27), although the prevalence of RAI reported in the literature in cirrhotics with stable disease varies between 7% and 83 % (9-11, 19-21, 27-32) depending on the methodology used for the diagnosis of adrenal insufficiency and the disease severity. Among the various methods for evaluating adrenal function, we chose the LD-SST because it has been shown to be more sensitive than SD-SST in non-critically ill cirrhotic patients (22). Using LD-SST, a normal response to stimulation is considered as a peak total serum cortisol of at least $18 \mu\text{g/dL}$ (22). We have chosen the cut-off value of $16 \mu\text{g/dL}$ in our study because it was shown to be more sensitive than $18 \mu\text{g/dL}$ used by other studies (26). There is no clear recommendation for the diagnosis of RAI in patients with stable liver cirrhosis. Although several studies have used the SD-SST, LD-SST was shown to be a more adequate and sensitive test for the diagnosis of RAI in cirrhotic patients without critical illness (11). We used the cutoff value of $16 \mu\text{g/dL}$ for peak total serum cortisol, proposed by several studies as a more appropriate cutoff for the diagnosis of RAI in patients with stable liver cirrhosis (11, 26). A meta-analysis including 11 studies (589 patients evaluated by LD-SST) found that values less than $16 \mu\text{g/dL}$ best predicted adrenal dysfunction (26). The frequency of RAI in our patients increased progressively with the severity of liver cirrhosis confirming previous data reported in other studies (11, 27, 29, 30, 33). Thus, Fede *et al.* (11) assessed RAI in a prospective observational study including 101 cirrhotic patients without sepsis or hemodynamic instability using LD-SST, and found a high prevalence (38%) of RAI, which was related to the severity of liver disease evaluated by CTP or MELD scores. RAI was defined by a peak total serum cortisol $< 18 \mu\text{g/dL}$ at 30 minutes after the injection of $1 \mu\text{g}$ of tetracosactrin. The same team assessed adrenal dysfunction in 79 patients with stable cirrhosis by measurements of serum total cortisol and serum free cortisol after LD-SST; adrenal dysfunction was defined by peak serum total cortisol $\leq 494 \text{ nmol/L}$ ($\leq 18 \mu\text{g/dL}$) and / or peak concentrations of free cortisol $\leq 33 \text{ nmol/L}$ ($\leq 1.2 \mu\text{g/dL}$) after the stimulation (34). The authors found that RAI was frequent in patients with

stable cirrhosis (34% when RAI was defined by peak total cortisol $\leq 494 \text{ nmol/L}$ ($\leq 18 \mu\text{g/dL}$), and 29% when RAI was defined by a peak free cortisol $\leq 33 \text{ nmol/L}$ ($\leq 1.2 \mu\text{g/dL}$). More recently, Fede *et al.* (20) assessed for the first time adrenal function in cirrhotic patients using adrenal responsiveness to prolonged ACTH stimulation. The authors used a multistep diagnostic approach: initially, a LD-SST was performed, followed by a long Synacthen test (cortisol concentrations measured before, 4, 8, and 24 hours after intramuscular injection of 1 mg tetracosactrin). Forty-six patients (38%) out of 121 patients with stable cirrhosis had RAI diagnosed by LD-SST, and 29 underwent the long Synacthen test of whom 55% showed a delayed cortisol response, suggesting that the mechanism of adrenal dysfunction in liver cirrhosis is related to a defect in hypothalamus-pituitary adrenal axis combined with a defective cortisol secretion at cellular level.

Jang *et al.* (33) prospectively evaluated the frequency of RAI defined as a delta cortisol less than $9 \mu\text{g/dL}$ (250 nmol/L) in noncritically ill patients at various stages of chronic liver disease using SD-SST and found that RAI was present in 13 (24.1%) of the 54 cirrhotics, but none (0%) of the 17 patients with chronic hepatitis; the prevalence of RAI among cirrhotics increased with increasing severity of liver disease from 0% in CTP-A to 57.1% in CTP-C. In another prospective study, Tan *et al.* (9) evaluated adrenal function using both serum total and plasma free cortisol in 43 clinically stable cirrhotic patients and 10 healthy volunteers. Cortisol levels were measured at baseline and following SD-SST, and RAI was defined by a delta cortisol of less than 250 nmol/L or a peak total cortisol below 500 nmol/L , or a peak plasma free cortisol $< 33 \text{ nmol/L}$. The prevalence of RAI was 39% using peak total cortisol $< 500 \text{ nmol/L}$, 47% using delta total cortisol $< 250 \text{ nmol/L}$, and 12% using plasma free cortisol $< 33 \text{ nmol/L}$. It is difficult to explain such significant differences in the rates of RAI diagnosis between the three diagnostic criteria used in this study. However, it should be mentioned that delta cortisol $< 250 \text{ nmol/L}$ (CIRCI criteria) for the diagnosis of RAI is recommended in critically ill cirrhotic patients (14), and the value of 33 nmol/L for peak plasma free cortisol has not been validated for diagnosis of RAI in liver cirrhosis (22). Of note, in contrast to our study and many others (29, 33), this study reported no association between the presence of RAI and the severity of cirrhosis. Galbois *et al.* (19) assessed the RAI in 88 hemodynamically stable cirrhotic patients using salivary cortisol as a marker of free cortisol concentration, and found RAI in 33% using

SD-SST, but in only 9.1% of cirrhotic patients when salivary cortisol was used to define RAI. Thevenot *et al.* (27) investigated the association between severity of cirrhosis and serum levels of total cortisol and free cortisol using LD-SST in a prospective study including 95 hemodynamically stable cirrhotic patients. The authors found that prevalence of RAI ranged between 7.4% and 49.4% according to the threshold used for the definition of RAI and that the mean values of baseline and 30 minutes after stimulation of serum total cortisol were 25% lower in CTP-C compared with CTP-A patients.

Our study has several limitations: single-center study, small number of patients included, and only serum total cortisol used for definition of RAI.

In conclusion, this study indicates that RAI defined by abnormal LD-SST is common in the setting of cirrhosis without hemodynamic instability or sepsis, and that it is related to the severity of the liver disease. High CTP and MELD scores, low serum HDL- and LDL- cholesterol levels may indicate the need for adrenal testing in cirrhotic patients. One future key goal would be to determine the clinical importance of RAI in patients with stable liver cirrhosis and, therefore, further studies are needed.

Conflict of interest.

The authors declare that they have no conflict of interest concerning this article.

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