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Assessment of adrenal function in patients with acute hepatitis using serum free and total cortisol

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ABSTRACT

Background: Adrenal dysfunction is frequently reported in severe acute hepatitis using serum total cortisol.
Aims: Because 90% of serum cortisol is bound to proteins that are altered during stress, we investigated the effect of decreased cortisol-binding proteins on serum total and free cortisol in severe acute hepatitis.
Methods: 43 severe and 31 non-severe acute hepatitis and 29 healthy controls were enrolled consecutively and studied prospectively. Baseline (T_0) and cosyntropin-stimulated (T_{60}) serum total and free cortisol concentrations were measured.
Results: T_0 and T_{60} serum total cortisol did not differ significantly between severe, non-severe hepatitis and healthy controls. Conversely, serum free cortisol (T_0 $p = 0.012$; T_{60} $p < 0.001$) concentrations increased from healthy controls to severe hepatitis, accompanied by a decrease in corticosteroid-binding globulin and albumin (all $p < 0.001$). In acute hepatitis ($n = 74$), patients with "low" corticosteroid-binding globulin (<28 mg/L) had higher T_0 serum free cortisol than others (103.1 [61.2–157] vs. 56.6 [43.6–81.9] nmol/L, $p = 0.0024$). Analysis of covariance showed that at equal concentration of total cortisol, the free cortisol concentration was significantly higher in severe than in non-severe hepatitis ($p < 0.001$) or healthy controls ($p < 0.001$).
Conclusions: In severe hepatitis, the decrease in cortisol-binding proteins impairs correct diagnosis of adrenal dysfunction. This could be corrected by measuring or estimating free cortisol.

1. Introduction

Acute liver failure (ALF) is a rare critical illness characterized by massive hepatic necrosis caused mainly by drug-induced liver injury [1]. The nonspecific clinical presentation of ALF may be easily confused with symptoms of acute adrenal dysfunction (AD), another life-threatening condition that could exacerbate ALF. AD typically arises in critical conditions such as severe infections, trauma or after aggressive surgery [2–4]. The excessive production of pro-inflammatory cytokines (IL-6 and TNF-α) found in ALF may account for the high mortality rate reported in this setting, and may also contribute to the occurrence of AD [5]. Indeed, pro-inflammatory cytokines may cause a shift from cortisol synthesis towards androgen production, or induce the dominant-negative

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β-isoform of the glucocorticoid receptor leading to AD [6,7]. Furthermore, it has been shown that IL-6 inhibited the synthesis of cortisol-binding globulin (CBG) [8] and low serum CBG concentrations have been reported in the early phase of severe burn injury and septic shock, two conditions that present high levels of IL-6 [9,10]. The decrease in the main cortisol-binding proteins (i.e. albumin or CBG, both synthesized in the liver) may lead to overestimation of the prevalence of AD when assessed by serum total cortisol (STC, i.e. bound plus free fractions), as previously reported in a cirrhotic population [10]. The assessment of AD using serum free cortisol (SFC) concentrations has been reported to correlate strongly with severity of illness [11].

Using STC, AD has been reported in 62% of ALF patients and could contribute to haemodynamic instability and mortality in these patients [12]. Corticosteroid replacement could be useful in ALF patients suspected to have AD, although this strategy remains conflicting in patients suffering from a septic shock with [13] or without cirrhosis [4,14]. Moreover, corticosteroids have been reported to be associated with an increased risk of shock relapse in cirrhotic patients with septic shock [13]. Therefore, the diagnosis of AD needs to be properly ascertained before starting any corticosteroid treatment.

We postulate that the high prevalence of AD reported in ALF [12] is misleadingly overestimated and should be corrected by measuring the free fraction of cortisol. The present CORT-HEPAT study aimed to assess, in patients with severe acute hepatitis (SAH), (i) variations in SFC and STC concentrations, and in cortisol-binding protein concentrations; (ii) correlations between STC and SFC concentrations; (iii) thresholds of SFC to identify patients with AD; (iv) agreement between measured SFC and estimated SFC using Coolens' or cubic equations [15,16].

2. Materials and methods

2.1. Study design and patient characteristics

This multicentre, prospective, observational study was conducted from August 2011 to February 2014 in the Hepatology Units of five university teaching hospitals in France (Besançon, Clichy, Villejuif, Lyon and Lille). The study was approved by the local ethics committee and patients provided written informed consent in accordance with the ethical guidelines of the Declaration of Helsinki. Inclusion criteria were consecutive patients aged between 18 and 75 years hospitalized for acute hepatitis defined as an abrupt rise in serum aminotransaminase levels during the previous 15 days (AST or ALT >500 IU/L or >10 times the upper normal value). Acute hepatitis was considered as severe if the prothrombin index was <50%, and as non-severe if the prothrombin index was >50%. We excluded patients with history of hypothalamic-pituitary or adrenal disease, chronic liver disease, corticosteroid treatment within the previous 6 months, ketoconazole intake, liver transplant recipients, acute alcoholic hepatitis and night workers. Twenty-nine healthy controls (HC) were also enrolled and were stratified with the SAH group in terms of age, gender and oestrogen intake, since oestrogen therapy was the most common cause of changes in CBG levels. To evaluate the range of SFC concentrations, eight patients with known AD caused by impairment of the hypothalamic–pituitary–adrenal (HPA) axis ($n = 5$) and adrenal gland ($n = 3$) followed at the Endocrinology Department of Besancon were also studied.

2.2. Laboratory measurements

STC and SFC concentrations were measured blindly before (T_0 between 8 am and 9 am) and 60 min after (T_{60}) intravenous injection of 250 μg of tetracosactrin (Synacthen®, Sigma-Tau laboratory, Issy-les-Moulineaux, France). Serum CBG, albumin and adrenocorticotropic hormone (ACTH) were also measured. Further details are provided in Appendix A.

2.3. Adrenal dysfunction assessment

We defined AD as a STC concentration <83 nmol/L (3 μg/dL) at T_0 or <550 nmol/L (20 μg/dL) at T_{60} [17]. In stressed SAH patients, we used the same criteria as those proposed by Harry et al., i.e. T_0 STC <250 nmol/L, T_{60} STC <500 nmol/L, or a delta STC level (i.e. the difference between cortisol values at T_{60} and T_0) <250 nmol/L [12]. Since the normal range of SFC concentrations in SAH patients remains uncertain, we used the range from 5th to the 10th percentile of the distribution of SFC to define abnormal cortisol values, assuming that none of SAH patients studied had AD. To determine the range of SFC values that may be used for AD diagnosis in unstressed patients, we used the maximum value of patients with known AD and the minimum value of HC.

2.4. Calculated serum free cortisol

We compared measured SFC concentrations using (1) Coolens' equation [15], and (2) the cubic solution [16] using the Bland and Altman method (see details in Appendix B).

2.5. Statistical analysis

Numerical variables are presented as mean ± standard deviation (SD) or as median and interquartile range [IQR] and categorical variables as number (percentage). The primary outcome was to compare STC, SFC and cortisol-binding proteins (CBG and albumin) concentrations between the three groups namely SAH, NSAH and HC. Continuous variables were compared using the Mann–Whitney test or the Student t test as appropriate. The Kruskal–Wallis test with Bonferroni's correction was used for within-group multiple comparisons and the Spearman correlation coefficient was calculated. We also compared SFC and STC concentrations in patients with "low" and "high" serum albumin or CBG concentrations. The cut-off values defining "high" and "low" concentrations of albumin and CBG were arbitrarily set at the 25th percentile. The strength of agreement between measured and calculated SFC was analyzed using the Bland–Altman method. An adequate free cortisol index (FCI) was defined as a ratio STC/CBG >12 after ACTH stimulation [18]. Finally, linear and quadratic regression models were fitted in order to study the relationship between STC and SFC within the three groups. Regression lines were compared between groups using covariance analysis, assuming that the hypothesis of homogeneous variance between groups was not rejected. Stratification variables (i.e. age, sex and oestrogen intake) were included in the multivariate model. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Patients characteristics

Seventy-five consecutive patients with acute hepatitis were enrolled, but one SAH patient was later excluded because he had received corticosteroids; 74 patients were included in the final analysis. Per protocol, the three groups (SAH = 43, NSAH = 31 and HC = 29) were proportionally distributed at baseline in terms of age, gender and oral contraception intake (details are reported in Table 1). In SAH patient, 8 patients (18.6%) had encephalopathy