Oral Glutamine Challenge Improves the Performance of Psychometric Tests for the Diagnosis of Minimal Hepatic Encephalopathy in Patients with Liver Cirrhosis

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ABSTRACT

Background & Aims. Minimal hepatic encephalopathy is difficult to diagnose due to the lack of a gold standard test. Oral glutamine challenge has been found to increase blood ammonia in cirrhosis leading to secondary cognitive impairment. The aim of this study was to evaluate the value of oral glutamine challenge in improving the psychometric performance for the diagnosis of minimal hepatic encephalopathy, and the risk of this condition for overt hepatic encephalopathy in cirrhotic patients.

Methods. Fifty-four cirrhotics (34 males; mean age 55.2 years) and 16 healthy controls were included. Minimal hepatic encephalopathy was assessed by the psychometric hepatic encephalopathy score. Arterial ammonia concentrations and psychometric tests were evaluated 60 minutes before and after a 20 g oral glutamine load. Follow-up lasted 12 months.

Results. At baseline, 29 (53.7%) of 54 patients had minimal hepatic encephalopathy and significantly more (79.63%) post-glutamine (p<0.0001). Baseline arterial ammonia levels significantly raised post-glutamine in cirrhotics (85.2±20.8µg/dL versus 159.82±66.01µg/dL, p<0.0001), while in controls they remained unchanged (p=0.064). For the diagnosis of minimal hepatic encephalopathy, baseline arterial blood ammonia showed an area under the ROC curve of 0.54 (CI95%: 0.402-0.680, p=0.58), with no significant post-glutamine changes (0.53, CI95%: 0.389-0.667, p=0.77). Ten patients (18.51%) developed overt hepatic encephalopathy, among which 9 had minimal hepatic encephalopathy (4 at baseline, 5 post-glutamine). At multivariate analysis, MELD score (1.5187, CI95%: 1.0690-2.1574, p=0.0197) was an independent predictor of the overt hepatic encephalopathy.

Conclusions. In cirrhotic patients, an oral glutamine load improves the psychometric diagnostic performance for minimal hepatic encephalopathy. MELD score has been independently related to overt hepatic encephalopathy.

Key words: minimal hepatic encephalopathy – oral glutamine challenge – psychometric test – ammonia.

INTRODUCTION

Hepatic encephalopathy (HE) is a serious neuropsychiatric complication of both acute and chronic liver failure, with a significant impact on the quality of life and a predictive value of poor outcome [1]. Although the origin of the toxins responsible for the altered mental has not been yet completely elucidated, the role of ammonia remains central, supported by a large number of studies [2-5]. In cirrhosis, the degree of liver failure and the presence of portosystemic shunts are responsible for increased levels of serum ammonia. However, hyperammonemia is difficult to evaluate in the context of HE, given the fact that correlations between ammonia levels and the severity of HE have proved to be extremely variable [6].

When cirrhotic patients are found normal under neurological clinical examination, but have mild cognitive and psychomotor deficits, the condition is referred to as minimal hepatic encephalopathy (MHE). Reaching a diagnosis of MHE can be difficult due to the lack of a gold standard test. Current methods for MHE diagnosis are subjective, timeconsuming, costly and hard to access. This might explain why most clinicians do not recommend testing for MHE in current clinical practice.

However, lately, some user-friendly screening tests for evaluating cognitive function in these patients have been proposed. Thus, Psychometric Hepatic Encephalopathy Score (PHES), a standardized test battery, has proved to be a short, objective, valid and reliable test for the assessment of MHE [7].

Oral glutamine challenge (OGC) is a method to increase blood ammonia in patients with cirrhosis, which could lead to cognitive disturbances. The aim of our work was to evaluate the role of OGC in improving the performance of psychometric tests for the diagnosis of MHE and the risk of this condition for progression to overt hepatic encephalopathy (OHE).

METHODS

Patients

This prospective study included 54 patients diagnosed with liver cirrhosis, hospitalized or followed-up in an outpatient clinic at the Gastroenterology and Hepatology Institute, "St. Spiridon" University Hospital, Iasi, Romania, between March 2010 and June 2011.

Inclusion criteria were: age > 18 years, normal neurologic signs upon examination, stable cirrhosis, grade 0 of HE (West-Haven criteria). Exclusion criteria were: OHE (grade 1 or higher), inability to perform psychometric tests, use of antibiotics, sedatives or lactulose in the previous 3 months, recent (<6 months) active alcoholism, diabetes mellitus, neurologic or psychiatric disorders, evidence of decompensated respiratory, cardiac and renal disease.

All patients underwent upper gastrointestinal endoscopy to detect the presence of esophageal varices. Arterial ammonia blood level assessment, together with the psychometric tests were performed pre- and post-glutamine load. Each patient was followed-up every 4 months over one year for development of OHE.

The study was conducted according to the provisions of the Helsinki Declaration and was approved by the local Ethics Committee. All subjects gave their written informed consent.

The control group consisted of 16 healthy subjects matched to patients according to age, sex, and education level.

Psychometric tests

After a thorough explanation of the tests, each patient and healthy subject performed the PHES at baseline and at 60 minutes after OGC. PHES includes number connection tests (NCT) A and B, digit-symbol test (DST), line tracing test (LTT) and serial dotting test (SDT). The results of PHES were determined using an online free calculator, available at http://www.redeh.org/TEST_phes.htm, based on the normality tables in the Spanish population. The diagnosis of MHE was defined by a PHES value \leq -5.

Oral glutamine challenge (OGC)

In a fasting state, patients and healthy control subjects ingested a 20g solution of glutamine (L-glutamine, Prolab Nutrition Inc. USA) dissolved in 100 ml tap water. Any symptoms that appeared during one hour were recorded. Samples of arterial blood (radial puncture) were taken at baseline and 60 minutes after glutamine load. Blood samples were immediately placed on ice and transported within 30 minutes to the laboratory for centrifugation and ammonia determination.

Data analysis

Statistical analysis was performed using Medcalc 12.3.0 and Microsoft Office Package. The results were expressed as means \pm SD. We used the receiver operating characteristics (ROC) curve in order to analyze the sensibility and the specificity of blood ammonia level (pre- and post-glutamine) as a tool for the diagnosis of MHE and for establishing optimal threshold values. Correlations between variables were examined with a Pearson correlation. Multivariate regression was performed for the determination of individual predictive factors for OHE.

RESULTS

The demographic and clinical characteristics of the patients are summarized in Table I.

Table I. Characteristics	of the studied patients
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Patients (n=54)	
Mean age, years (range)	55.2±7.6 (42-63)
Gender (M / F)	34 / 20
Etiology of cirrhosis	
Alcohol	34
HCV	16
HBV	2
Cryptogenic	2
MELD score (range)	9 (7-19)
Child-Pugh class (A/B/C)	31/19/4
Esophageal varices (grade I/II/III)	8/10/12

Ammonia blood levels

Post-glutamine load, a significant raise of arterial ammonia levels in patients with cirrhosis (85.2 ± 20.8 versus 159.82 ± 66.01 µg/dL, p <0.0001) was observed, while in healthy control subjects the changes did not reach the level of significance (47.15 ± 17.3 µg/dL versus 52.15 ± 18.07 µg/dL, p =0.064).

Using the ROC curve analysis, cut-off values of ammonia blood levels both at baseline and post-OGC were defined (87.8 μ g/dL and 124 μ g/dL, respectively). For the diagnosis of MHE, baseline blood ammonia showed an area under the ROC curve (AUROC) of 0.54 (CI: 0.402-0.680, p= 0.58), while the post-OGC was 0.53 (CI: 0.389-0.667, p=0.77) (Fig. 1 A,B).

Prevalence of MHE before and after OGC

At baseline, 29 of 54 patients (53.7 %) met the PHES criteria for MHE diagnosis. After glutamine load, the percentage of patients diagnosed with MHE increased to 43 (79.6 %). The values of PHES were significantly lower post-OGC compared to baseline (P<0.0001), suggesting that OGC increased the diagnostic performance of PHES for MHE in cirrhotic patients, and it remained almost unchanged in healthy subjects (Fig. 2).

An altered OGC defined by ROC curve as an increase of ammonia over 124 μ g/dL post-glutamine load was found in 37 patients (68.51%). Among these, 30 (81.1%) had MHE, while 7 (18.9%) did not have MHE.



Fig. 1. ROC curves showing the sensitivity and specificity of baseline (A) and post- glutamine load (B) arterial blood ammonia levels for the diagnosis of MHE.



Fig. 2. PHES scores at baseline and post-glutamine load in cirrhotics (PHES and PHES_Glut, respectively) and controls (PHES_c and PHES_c_Glut, respectively).

Oral glutamine challenge was well tolerated both by cirrhotic patients (only 1 had nausea) and by healthy subjects (1 complained of abdominal discomfort). No patient developed clinical neurological signs of OHE after glutamine load.

Incidence of overt HE during the follow-up

On follow-up, 10 patients (18.51%) developed OHE. Among these, 9 had MHE (4 at baseline and 5 after glutamine load), and 1 patient met no criteria for MHE both at baseline and post-glutamine.

The development of OHE was significantly associated with the post-glutamine PHES score (n= 54, r= -0.382, p= 0.004),

The following variables were considered in a predictive model for OHE: Child and MELD scores, grade of esophageal varices, pre- and post-glutamine arterial blood ammonia. In the multivariate regression analysis only the MELD score was an independent predictive factor for the development of OHE (OR = 1.5187, 95% CI: 1.0690 - 2.1574, p = 0.0197).

DISCUSSION

Hepatic encephalopathy is the late effect of portal hypertension with a high grade of porto-systemic shunts, spontaneous or surgically created. Minimal HE has no specific clinical expression, thus being hard to assess. Diagnosis of MHE is still controversial, especially when it comes to establish criteria and reliable diagnostic tests easily applied in clinical practice. Neurological and psychological evaluations do not offer sufficient clinical tools for diagnosing MHE and assessing its progression to OHE.

In the present study, we found that glutamine load increases the performance of psychometric tests (PHES) for MHE diagnosis in patients with liver cirrhosis. Previous studies have reported that glutamine-induced increased levels of ammonia were associated with variable modifications of psychometric testing [8-11]. Thus, Oppong et al [9] showed that glutamine did not influence NCT, but modified the choice reaction time. In another study [11], increased level of ammonia following glutamine load did not change psychometric testing results for attention, orientation and memory. Douglass et al [12], using a combination of aminoacids with a hemoglobin-like composition, created a model of episodic encephalopathy and showed significant changes in ammonia concentration as well as in psychometric and electroencephalographic testing. Increased concentrations of ammonia were also reported in patients with cirrhosis Child A, without inducing any modifications in the psychometric testing performance [13]. The differences in psychometric testing results throughout reports in the literature could be explained by variations in the type of test used, diagnosis criteria, and the type and dose of glutamine. Using the battery of psychometric test PHES, which is accepted as a standard diagnostic test for MHE in several countries [14-18], we showed that it represents an efficient tool in the evaluation of cirrhotic patients with mild cognitive deficits.

Glutamine was administrated at a dose of 20 g, similar to that in some studies [10], but higher than what was being used in others [8,19]. However, this dose was well tolerated and did not precipitate OHE in our cirrhotic patients. In line with previous reports [8, 10, 11], glutamine load did not modify the psychometric testing results and ammonia levels in the controls, demonstrating that it is not implicated in the cognitive status of the healthy subjects.

In our study, the prevalence of MHE at baseline was 53.7%, which is concordant with previous studies [15, 20, 21], but higher than in other studies that included patients with mild or moderate hepatic disease [22, 23]. Ditisheim et al [10] reported a prevalence of 44% for MHE in patients with moderate or severe hepatic failure. These differences suggest the implication

of multiple risk factors in MHE, besides the severity of hepatic failure [22]. Thus, alcoholic etiology of cirrhosis in 62.96% of cases and large esophageal varices (3rd degree) in 22.22 % of patients could be major risk factors for MHE in our study.

We decided to perform OGC using arterial blood ammonia as, according to previous studies [24-26], it proved more relevant than the venous one in the assessment of HE. Our study showed that arterial ammonia does not represent a specific biological marker for the diagnosis of MHE. Moreover, glutamine does not improve its diagnostic performance, as showed by the AUROC value (0.54 at baseline and 0.53 postglutamine load).

It seems that assessing ammonia level in capillary blood could be a simpler and more convenient alternative to arterial determination [27, 28]. Thus, in patients with MHE, Ditisheim et al [10] showed increased capillary ammonia levels following OGC versus basal ammonia, the AUROC value rising after the glutamine load from 0.541 to 0.727.

In the present study, the incidence of OHE on the followup was 18.51%, a result in line with some previous reports [8]. The risk for OHE development was significantly higher in patients with MHE than in those without MHE. This fact confirms previous data regarding the natural history of MHE and its role in the progression to OHE [29].

Post-glutamine PHES score, unlike PHES alone, was significantly associated with the development of OHE (p= 0.004), showing that OGC increases its prognostic value. We have also found that the MELD score was a predictive factor with an independent value for OHE development. Considering the increase in both diagnostic performance and prognostic value of post-glutamine PHES, this test could be a useful and better screening tool for MHE than PHES alone. However, its feasibility is limited by the lack of convenience and time constraints.

Our study has several limitations such as the relatively small size of the samples, especially of patients with severe liver disease. Since PHES evaluation has not yet been standardized in our country, we have used a standardized PHES scoring based on Spanish norms which could influence our classification. However, the presumed differences between norms of other countries were limited by the use of a control group. Future studies should definitely move towards finding a larger applicable set of norms for PHES scoring in representative samples.

CONCLUSIONS

In cirrhotic patients, an oral glutamine load improves the performance of psychometric tests for the diagnostic of MHE, a condition which has proved to have a high risk for development of OHE. In addition to MHE, MELD score is an independent predictive factor for the development of OHE.

Conflicts of interest: None to declare.

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