Original Article

EFFECTIVENESS OF ORAL GLUTAMINE CHALLENGE TEST IN DIAGNOSING MINIMAL HEPATIC ENCEPHALOPATHY IN LIVER CIRRHOSIS PATIENTS

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ABSTRACT

Background: Due to the lack of a gold standard test, it is difficult to diagnose minimal hepatic encephalopathy. Oral glutamine challenge has been found to increase blood ammonia in patients with cirrhosis. This study was aimed to investigate the accuracy of the oral glutamine challenge test in diagnosing minimal hepatic encephalopathy

Material and Methods: This validation **s**tudy was performed at the gastroenterology unit, East Medical Ward, Mayo Hospital, Lahore, from September 2016 to November 2018 by using the non-probability convenience sampling technique. All patients included in the study had undergone a baseline blood ammonia measurement and were administered Trail Making Test-A and B; those having an abnormal test were diagnosed to have minimal hepatic encephalopathy (MHE). Arterial blood samples were collected for ammonia measurement. After that, patients were given oral glutamine challenge and blood ammonia levels were again determined after 60 minutes, together with psychometric tests.

Results: The mean age of study participants was 46.22±10.04 years. Psychometric Test-A showed positive findings in 50 patients. Psychometric Test-B showed the same findings as that of Psychometric Test A. Mean ammonia level before Oral glutamine challenge (OGC) was 88.71±45.20 mg/dL. After the OGC test, the mean ammonia level was 145.16±69.02 mg/dL. The sensitivity and Specificity of OGC to diagnose MHE was 86% and 93.26% respectively. While 87.75% value represents positive prediction and a value of 92.22% suggests negative prediction. Overall diagnostic accuracy of OGC for detecting MHE was 90.65%.

Conclusion: In liver cirrhosis patients, the oral glutamine challenge test is as effective as a psychometric test for the diagnosis of the MHE.

Key Words: **A**mmonia, Hepatic encephalopathy, Glutamine

INTRODUCTION

Liver diseases are one of the major causes of mortality worldwide.[1] Hepatic encephalopathy (HE) is a neuropsychiatric dysfunction that can ensue in patients with liver disease, excluding other primary neurological disorders. The minimal hepatic encephalopathy (MHE) presents with the neurocognitive disorder of mild intensity and is present in patients having liver cirrhosis with or without porto-systemic shunts.[2,3]

MHE is often prevalent in patients having liver cirrhosis. The prevalence of MHE has been estimated between 20% to 74% in patients with liver cirrhosis, but the gold standard test for diagnosis has not been yet established.[4,5] Diagnosis of MHE is very important as it may affect daily life.[6,7] Oral administration of an amino acid solution categorically prepared to be similar to the amino acid composition of hemoglobin in cirrhotic patients leading to an incrimination in brain glutamine and dihydrogen monoxide levels. This process results in the deterioration of neuropsychological attainment. Altered glucose metabolism and cerebral perfusion caused by changes in ammonia concentration are associated with decreased utilization of glucose by sundry

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cortical areas concerned with cognitive functions.[8,9] MHE diagnosis reposes on the substantiation of illness responsible for MHE, like cirrhosis and portosystemic shunt, omission of normal brain functions as per clinical assessment, presence of un-coordination and neurophysiological variables, and omission of concurrent neurological dysfunctions. The objective of the OGC test is to quantitatively assess any increase in blood ammonia post glutamine oral intake, simulating oral protein intake during meals.[10] The assessment is carried out in the morning after at least 12 hours of fasting. An intravenous cannula is inserted followed by oral administration of either 10 or 20 g glutamine mixed in 100 ml of water. Twenty grams of glutamine contains 3.8 g nitrogen suggesting ingestion of approximately 24 grams of protein. A total of four blood samples are collected regularly each at half an hour interval after ingestion of glutamine to assess levels of ammonia in the blood.[11] It is anticipated that oral ingestion of glutamine will enhance blood ammonia in patients with the impending condition of liver cirrhosis but a healthy individual serving as control will not exhibit a similar upward trend of blood ammonia. The level of ammonia in blood after 1 hour of oral ingestion of glutamine is expressed as an ammonia response. The ammonia level appears to peak at between half an hour to one hour after ingestion, suggesting its association with the post-ingestion metabolism of glutamine in the small intestine. A less than 75 mg/dl level of blood ammonia is considered below the threshold for a positive test while higher than 128 mg/dl at one-hour post ingestion is typically established as pathological response.[12] Patients with disturbed OGC and MHE are appeared to be more vulnerable with the risk of developing overt HE (OHE) during follow-up compared to patients with typical OGC and having no MHE. This proposes that an altered value of OGC could function as a predictive factor for developing OHE in patients with MHE.[13-16] The current study aims to investigate the accuracy of the oral glutamine challenge test by measuring the ammonia levels in the blood of patients having liver cirrhosis due to viral hepatitis B and C.

MATERIAL AND METHODS

This validation study was conducted at the gastroenterology unit, East Medical Ward, Mayo Hospital, Lahore, from September 2016 to November 2018 using a non-probability convenience sampling technique. Keeping confidence level at 95% and margin of error at 9%, the sample size of 139 patients was determined by Rao soft sample size calculator. Informed consent was taken from study participants. All patients were enrolled after the diagnosis of compensated liver cirrhosis due to viral hepatitis B or C, confirmed by clinical and biochemical methods and radiological assessments.

Basic demographic characteristics like age, gender, etc. were recorded. Investigations including recent blood profiles i.e., complete blood count, renal function tests, liver function tests, activated partial thromboplastin time, international normalization ratio, ultrasound abdomen, and viral markers were also performed. The patients included in the study were invited to undergo a baseline blood ammonia measurement and were administered Trail Making Test-A and B, those with the abnormal test were diagnosed to have MHE. The arterial blood sample was drawn for ammonia measurement and samples were transported to the laboratory immediately in an ice bag. Measurement of ammonia levels was performed within one hour of sampling. Following this, patients were given oral glutamine challenge (20 grams of glutamine in 50 mL distilled water) and blood ammonia levels were again determined at 60 minutes, together with psychometric tests.

Data were analyzed using SPSS version 20 (IBM SPSS, 2016). Descriptive summary analyses were performed for calculating means and standard deviations for continuous variables and frequencies for categorical variables. Blood ammonia levels of patients with MHE were compared to

those without MHE. Results of the oral glutamine test were concluded by calculating positive and negative predictive values, sensitivity, and specificity of the test to determine the accuracy.

RESULTS

The mean age of participants was 46.22±10.04 years. The maximum and minimum ages of the patients were 70 and 19 respectively. Gender distribution of patients showed that there were 44(31.7%) female and 95(68.3%) male patients. The result of psychometric Test-A was positive in 50 patients while 89 patients exhibited negative results of the test. Psychometric Test-B showed similar findings as of Psychometric Test A with positive findings in 50 patients. The mean ammonia level before OGC was 88.71 mg/dl ranging between 47 and 177 mg/dl. After the OGC test, mean ammonia levels were 145.16±69.02 mg/dl. At this point, maximum and minimum ammonia levels seen in patients were 271 mg/dl and 88mg/dl respectively. The diagnostic accuracy of OGC was determined while keeping the Psychometric test-A as a gold standard test. Specificity and sensitivity of OGC for diagnosis of MHE were recorded as 93.26% and 86% respectively. While positive and negative predictive values of OGC were 87.75% and 92.22% respectively. Overall, the diagnostic accuracy of OGC for detecting MHE was 90.65%. (Table-1)

Table-1: Diagnostic accuracy of OGC test while taking psychometric test-A as the gold standard

		Test-A		Total
		Positive	Negative	
OGC	Positive	43	6	**49**
	Negative	7	83	**90**
Total		**50**	**89**	**139**

Sensitivity= 86%
Specificity= 93.26%
Positive Predictive value= 87.75%
Negative Predictive value= 92.22%
Diagnostic Accuracy= 90.65%

DISCUSSION

Hepatic encephalopathy is one of the signs of portal hypertension with spontaneously created high-grade portosystemic shunts. There are no specific clinical manifestations of minimal hepatic encephalopathy making it difficult to diagnose. Presently, the diagnosis of MHE is contentious, especially regarding establishing criteria and robust diagnostic assessment that is robust and readily applicable in clinical practice. Psychological and neurological assessments often do not present enough clinical evidence for MHE diagnosis. The present study attempted to assess the diagnostic accuracy of the OGC test compared to the psychometric test for MHE diagnosis. The results demonstrated that the diagnostic accuracy of OGC for the diagnosis of MHE was 90.65%. In addition, other diagnostic accuracy parameters like negative and positive predictive values, specificity, and sensitivity of OGC were 92.22%, 87.75%, 93.26%, and 86% respectively. There is no single gold standard test for diagnosis of MHE, a combination of two neuropsychological tests/psychometric hepatic encephalopathy score battery tests or neurophysiological tests is standard for diagnosis of MHE in liver cirrhosis in absence of overt encephalopathy.[15] Various combinations of psychometric tests with or without neurophysiological methods are required for the diagnosis of MHE in liver cirrhosis in absence of overt encephalopathy.[16]

Findings of this study showed that the mean ammonia level before OGC was 88.71 mg/dl ranging between 47 and 177 mg/dl. After the OGC test, mean ammonia levels were 145.16±69.02 mg/dl, indicating a significant rise in ammonia level. Ditisheim et al. in agreement with the present study, reported a rise in capillary ammonia levels comparing OGC induced ammonia with basal levels to rise from 0.541 to 0.727 in AUROC value after 60 minutes of glutamine ingestion.[17]

In this study, in all participants with liver cirrhosis, ammonia levels raised after the OGC test (145.16±69.02 mg/dl). Romero-Gómez et al assessed the usefulness of oral

glutamine challenge (OGC) and minimal hepatic encephalopathy in evaluating the risk of overt hepatic encephalopathy in cirrhotic patients. They revealed that patients with higher than 128 mg/dl of blood ammonia level measured for OGC indicate pathological condition. In healthy controls, ammonia concentrations remained unchanged but increased significantly in cirrhotic patients (127.43±78.6). In multiple logistic regression analysis, altered OGC was related to MHE (OR=5.45; 95% CI=1.17–25.4).[18]

Ampuero et al also described that oral glutamine challenge induces an increase in blood ammonia in patients with cirrhosis but not in healthy control or transplanted subjects. Oral glutamine challenge can predict minimal hepatic encephalopathy, as well as is associated with poor survival.[14]

According to the findings of this study, the diagnostic accuracy of the OGC test is 90.65% indicating the usefulness of this test in the diagnosis of MHE. Results of another study conducted by Irimia et al demonstrated that arterial ammonia levels significantly increased in post-glutamine ingestion (85.2±20.8μg/dL to 159.82±66.01μg/dL) compared to control where it remained unchanged. At baseline, 53.7 % of patients met the Psychometric Hepatic Encephalopathy Score (PHES) criteria for MHE diagnosis. After glutamine load, the percentage of patients diagnosed with MHE increased to 79.6 %. The values of PHES were significantly lower post-OGC compared to baseline, suggesting that OGC increased the diagnostic performance of PHES for MHE in cirrhotic patients, and it remained almost unchanged in healthy subjects.[10]

Some other researchers also emphasized the need for standard screening and diagnostic tests for MHE. In their opinion, the fact that MHE can affect daily life even though patients are asymptomatic signifies the need for screening tests. These tests should be easy to use, and quantifiable in a short time.[19] The oral glutamine challenge test is relatively simple and poses no adverse implications to the patients. The present study in conjuncture with previously published literature confirms that the OGC test can predict the MHE. Results of this study also suggest that future research looking into developing potential drug inhibiting activity of glutaminase enzyme could be promising to treat patients with MHE.

CONCLUSION

It is concluded that the diagnostic accuracy of OGC for the diagnosis of MHE is 90.65%. Negative and positive predictive values, specificity, and sensitivity of OGC are 92.22%, 87.76%, 93.26%, and 86% respectively. An oral glutamine challenge test is as effective as psychometric evaluations for the diagnosis of MHE in liver cirrhotic patients.

AUTHOR'S CONTRIBUTION

ROF: Primary topic idea
FH: Data analysis
SL: Critical review
UC: Helping data collection
TW: Peer and critical review

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