Small Intestinal Bacterial Overgrowth is not Associated with Minimal Hepatic Encephalopathy in Patients with Liver Cirrhosis

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Abstract

Background and Aim: Bacterial ammonia is considered to play a significant role in the pathogenesis of (minimal) hepatic encephalopathy ((M) HE). The urease activity of intestinal bacteria is recognized as a relevant factor. This has led to therapeutic strategies based on non-resorbable antibiotics and non-resorbable disacchararides. The role of small intestinal bacterial overgrowth (SIBO) is still unclear whereby recently published studies revealed a significant association between the prevalence of SIBO and MHE.

Patients and Methods: 52 consecutive patients with liver cirrhosis underwent MHE testing by critical flicker frequency analysis and testing for SIBO with the H_2 glucose breath test (H_2BT).

Results: The prevalence of MHE was 38.5% whereas the prevalence of SIBO was 0% in patients suffering from MHE and 3.2% in cirrhotic patients without MHE.

Discussion and Conclusion: Non-invasive tests for SIBO do not detect dysbalances in the microbiota of the gastrointestinal tract involved in the pathogenesis of MHE. Molecular technique-driven approaches are needed to identify distinct changes in the bacterial composition in patients with (M) HE.

Keywords: Minimal hepatic encephalopathy; Small intestinal bacterial overgrowth; Liver cirrhosis; Hepatic encephalopathy

Introduction

The pathogenesis of hepatic encephalopathy (HE) is still not completely understood. This frequent complication of cirrhotic patients leads to clinical or subclinical (minimal hepatic encephalopathy, MHE) reversible brain function disorders significantly impacting on the quality of life of affected patients. The clinical stratification of hepatic encephalopathy (HE) is enabled by the West Haven Criteria. MHE is the clinically inapparent presentation of HE, and its diagnosis can just be made by psychometric and neurophysiological tests. All grades of HE are estimated to be present in 30% to 45% of patients with liver cirrhosis (LC) and approximately 60% to 80% of cirrhotic patients yield evidence of cognitive dysfunction in specialized testing. All clinical manifestations of HE are defined to occur without any structural cerebral changes [1,2].

Driven by the therapeutical effects of non-absorbable antibiotics and non-absorbable disaccharides ammonia produced by intestinal bacteria is considered to be the most relevant factor in the pathogenesis of HE. In patients with liver cirrhosis the detoxification capacity of the liver is impaired. This leads to increased blood levels of these substances and, in the case of molecules able to pass the blood–brain barrier, to increased cerebrospinal fluid levels. Morphological changes presented as low-grade cerebral edema lead to alterations of neurotransmitter receptor systems and changes of the synaptic plasticity [3].

In addition to ammonia, other factors may be involved in the pathogenesis of HE as well: mercaptans, aromatic amino acids, short- and medium-chain fatty acids and endogenous benzodiazepines. Intestinal bacteria influence the luminal intestinal concentrations of short chain fatty acids and are considered to be a relevant source of ammonia in humans.

Dysbiosis of the intestinal bacteria appears to be involved in the pathogenesis of hepatic

encephalopathy. Small intestinal bacterial overgrowth is a frequent but frequently under diagnosed quantitative disturbance of intestinal microbiota with unclear prevalence. Different predisposing conditions have been recognized, including age, chronic pancreatitis, conditions of suppressed immune functions, Celiac disease, Crohn´s disease and the broad spectrum of anatomical disorders of the gut including postoperative loss of ileo-coecal continence. Different studies demonstrated an association of liver cirrhosis with SIBO explained by alterations of the gut microbiota and a slow intestinal transit time due to portal hypertension [4-6]. In healthy individuals the small bowel is colonized by a small number of bacteria in a stable composition with concentrations of 10^3 - 10^4 CFU/ml. SIBO is characterized by an abnormally increasing number of CFU of bacteria (up to 10^6/ml) in the small bowel. The variety of clinical symptoms and presentations is broad and includes abdominal symptoms such as fullness, pain, diarrhea, meteorism and flatulation as well as symptoms of malabsorption followed by loss of body weight and decreased thrive. Diagnostic algorithms include aspiration of jejunal liquids and culture depending methods. Besides invasive and cost-consuming limitations these tests are restricted to a narrow spectrum of cultivable intestinal bacteria. In clinical routine glucose hydrogen breath test (H_2BT) is the most common test. The test is based on the quantification of hydrogen in the exhaled air as a product of hydrogen producing bacteria after carbohydrate fermentation. The test is not standardized but has clear advantages over invasive tests: safe, reproducible, cheap, easy to perform and broadly available.

Material and Methods

Between October 2013 and April 2015, a total of 52 consecutive cirrhotic patients (male n= 48, female n=4) cared for in the outpatient department of the Department of Gastroenterology, Hepatology and Infectious Diseases of the University of Magdeburg, Germany, were enrolled. The study protocol was performed in accordance with current GCP guidelines and the declaration of Helsinki and approved by the local ethic committee of the University of Magdeburg (study identification number of local ethic committee 177/12). All patients gave their written informed consent to participate in the study. Demographic characteristics are described in Table1.

The collected epidemiological and clinical data include sex, age, etiology of cirrhosis, data on previous *H. pylori* eradication therapy, co-morbidities, especially diabetes mellitus, previously prescribed antibiotic therapies and ongoing medical therapy including any therapies addressing HE.

The most relevant exclusion criteria were antibiotic therapy during the last 4 weeks, ongoing therapy for (M) HE, axis deviation of the eyes, color blindness, inability to take part in the neurophysiological and psychometric tests and presence of overt HE.

All patients underwent laboratory, psychometric und neurophysiological testing as described below.

Glucose H₂ breath test

After an overnight fast, H_2 breath test was performed between 08:00 and 09:00 am by a trained and highly specialized technician in a standardized manner. Dietary recommendations for all patients included to generally avoid the intake of complex carbohydrates 3 days before the test.

All patients received 50 g D-glucose (Merck AG, Darmstadt, Germany) which was solved in 200 ml of water and had to be drunken within 3-5 minutes.

Breath samples were collected every 10 minutes within the first hour, every 20 minutes during the following other two hours.

Breath samples were analyzed for hydrogen concentration as parts per million (ppm) by an electrochemical analyzer (Gastrolyzer, Bedfont Scientific Ltd., Harrietsham, UK). According to the recommendation of the German society of digestive and metabolic diseases (DGVS) [7] small intestinal bacterial overgrowth was diagnosed if the breath hydrogen content exceeded 20 ppm compared with the baseline value.

Ammonia measurement

Fasting venous blood samples were obtained from all patients on the day of psychometric testing. The ammonia concentration was measured in µmol/l (normal range 11.2 – 55.3) according to the manufacturer's instructions.

Psychometric diagnostic procedures for MHE

Number connecting test (NCT): The NCT-A was performed by a medical doctor or student to detect MHE. The patients were required to connect increasing numbers from 1-25 printed on a paper, without interrupting but as quickly as possible. A pathological finding was defined if more than 30 seconds were needed to complete this task.

Critical flicker frequency (CFF) analysis: For the evaluation of MHE the HEPAtonorm ™ Analyzer (HE Flicker Diagnostics GbR, Düsseldorf, Germany) was applied. The concept of intrafoveal stimulation with a luminous pulsating light emitting diode requires an accurately lens system with unimpaired eye accommodation. A continuously gradual decreasing frequency of a light impulse, starting with 60 Hz and stopping with 25 Hz, is stopped by the patients in case of the impression of a flickering light. Using a cut-off value of ≥39 Hz, ten measurements were performed and the mean value was calculated after a brief instruction and training period.

All measurements were performed between 8:00 am and 12:00 am in a quiet room with constant light conditions.

Statistical Analysis

A database in Excel 2010 (Microsoft Corporation, Redmond, Wash., USA) was created from medical records. All statistical analyses were performed using IBM SPSS Statistics 21.0.0 (IBM Corporation, New York, N.Y., USA). Results for numerical data are given as mean with standard deviation. For categorical data, results are given as absolute numbers with percentage. For comparison of categorical data, chi square test was applied if the expected incidence exceeded 5; otherwise Fisher´s exact test was used. T tests (welch test, satterthwaite's approximation to compute the degree of freedom) were used for testing homogeneity of independent samples in continuous data. All statistical decisions were made two tailed with a critical probability of α= 0.05 without α adjustment.

Results

A total of 52 patients (92% male) with confirmed liver cirrhosis were screened for MHE and SIBO. All of these patients had no past medical history for previous episodes of overt HE or had been diagnosed with MHE in the past, and no patient had a history of *H. pylori* eradication. 52% (n= 27) of the patients were suffering from alcoholic liver cirrhosis, 12% (n= 6) from non- alcoholic - steatohepatitis (NASH) - cirrhosis and 14% (n= 7) presented more

Table 1:

	N
Gender M:F	48: 4
Age	66, 9 years ± 9,2
Child – Pugh- stage	
A	37
B	13
C	2
Etiologyoflivercirrhosis	
Alcohol	27
NASH	6
> 1 riskfactor	7
HCV	4
HBV	1
Other causes	4
unknown	3
Ammonia (µmol/l)	47.7 6.8- 369.8 ± 64
Bodyweight (kg)	84.5 62-130 ± 16.1
Size (cm)	177.8 160- 196 ± 7.2
BMI (kg/m²)	26.7 21.1 – 38 ± 4.3

Table 2: Prevalence of MHE using CFF in different stages of liver cirrhosis.

	MHE n (%) CFF
Liver cirrhosis	20 (37,5)
Child A	14 (37,84)
Child B	4 (30,77)
Child C	2 (100)

than one risk factor, e.g. alcohol abuse and metabolic risk profile. In 23% (n= 12) cases other causes of chronic liver disease were diagnosed. The stages and the etiology of liver disease are summarized in Table 1. 71% of all patients had no significant impairment of liver function and where therefore in Child- Pugh- Stage A.

In our cohort 38, 5% (n=20) of the cirrhotic patients suffered from MHE defined by the results of CFF (< 39 Hz). Considering the stage of liver disease, the prevalence of MHE was 37, 8% in Child A (n= 14), 30, 8 % in Child B (n= 4) and 100% in Child C patients (n = 2) Table 2.

The Glucose H_2 Breath Test was positive in only one patient without MHE suffering from liver cirrhosis Child B. Any other tested patient did not present evidence for MHE using this test.

Discussion

Based on the fact that ammonia is the best recognized factor in the pathogenesis of HE we postulated a role of SIBO in the pathogenesis of (M) HE. The assumption of SIBO as a responsible mechanism for increased blood ammonia levels has not been confirmed in our study. Previously published data had shown the contrary with an increased prevalence of SIBO in cirrhotic patients. Pande and colleagues reported the prevalence of SIBO in 49 % of cirrhotic patients compared to 8 % in controls using glucose- hydrogen breath test [8]. Our data differ significantly from the results of this trial conducted in India. Likely explanations are different intestinal bacterial compositions in India and Germany as a consequence of different dietary habits that might result in a larger proportion of patients that are H_2 non-producers. Differences between the test equipment used for the assessment could be another explanation. As a meaningful difference the Indian group used 100 g glucose dissolved in 200 ml water compared to 50 g in our study, and the Child- Pugh- stages (A= 29%, B= 45%, C= 26%) of the patients differ significantly from our cohort (A= 71.2%, B= 25% C= 3.8%). The different amount of glucose might influence the sensitivity of the Glucose H_2 Breath- test. German guidelines recommend the use

of 50 g solved in 200 ml water glucose Glucose H_2 Breath- test. Studies comparing different amounts of glucose do not exist but different amounts in a range from 50- 80 g glucose are used. In accordance to widely published data we decided to use a dose of 50 g glucose [9-12].

In addition, more severe stages of liver cirrhosis may result in a higher prevalence of SIBO in this cohort. A recently published work from a Chinese group detected a prevalence of SIBO in 33 % of cirrhotic patients and 85% (n= 17) of these patients suffered from MHE [13]. The authors did not report the methodology of the breath test as well as the characteristics of their cohort regarding the severity of the liver cirrhosis.

Our results allow several conclusions: assuming SIBO is a relevant complication of liver cirrhosis H_2BT is not a suitable test to detect this bacterial dysbalance in caucasian patients. Considered to be the gold standard aspiration of intestinal content and use of culturing techniques should be re-evaluated in the near future with the opportunity of new nucleotide sequencing techniques and new biocomputational tools with the ability to overcome low count of culturable intestinal bacteria [14–17]. Several studies demonstrated differences in the fecal microbiota using culture- independent techniques comparing patients with liver cirrhosis with controls and patients with overt HE with MHE and controls or changes in the bacterial community after therapy [18–22]. In the majority fecal samples were analysed tolerating the disadvantage of missing assignment of the bacterial DNA to intestinal locations. Interestingly significant differences between sigmoid colonic mucosal microbiome and fecal microbiome in intra-subject analysis were detected. This emphasizes the idea of more proximal changes in the intestinal microbiome in cirrhotic patients.

Modulation of the intestinal microbiota composition by different means is proven to prevent and treat HE in cirrhotic patients. Probiotics demonstrated to be effective in prevention of HE in prospective randomized trials [23,24] whereas watch and wait was associated with significant higher rates of recurrence of HE. Lactulose as non- resorbable dissacharide is the competing therapeutical approach in treatment of HE. Non-resorbable antibiotics as Rifaximin are the new concept in preventing and treating HE episodes [25–28]. Addressing a reduction of the gut ammoniagenesis caused by bacteria both concepts (lactulose and Rifaximin) support the theory of a dominant role of bacterial synthesized ammonia in the pathogenesis of HE.

Conclusion

Despite the fact that intestinal bacteria dysbiosis and SIBO are assumed to be a major component in the pathogenesis of MHE we did not detect a significant prevalence of SIBO as diagnosed by H_2 breath test in our cohort of patients with liver cirrhosis and MHE. Further studies employing molecular analyses of intestinal bacteria are needed to identify changes of the bacterial composition in the intestinal lumen in particular.

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