Prevalence of *Helicobacter pylori* Infection in Patients with Minimal Hepatic Encephalopathy

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**ABSTRACT**

**Background:** Ammonia is a critical factor in the pathogenesis of minimal hepatic encephalopathy (MHE). Most of the ammonia is from bacterial production in the intestinal tract. Non-absorbable antibiotics and non-absorbable disaccharides are therefore the therapy of choice. A few studies have addressed the effect of ammonia produced by *H. pylori* in the pathogenesis of MHE.

**Methods:** In this prospective clinical trial, 84 consecutive patients with liver cirrhosis (LC) underwent laboratory, psychometric und neurophysiological testing to determine serological *H. pylori* status, MHE and blood ammonia levels. Relevant clinical and demographic characteristics were documented.

**Results:** Out of 84 LC patients (83% male), 29% presented with MHE as assessed by critical flicker frequency analysis (CFF). The prevalence of *H. pylori* infection in the cohort was 21%; 22% of *H. pylori*-infected patients presented with MHE according to the criterion of a positive CFF result. If the criterion for MHE was a positive CFF and a positive NCT-A result, then 17% of *H. pylori* positive patients suffered from MHE. The prevalence of MHE in *H. pylori*-negative patients, based on CFF alone and on the combination, was 30%. A proportion of 19% of the patients with MHE had increased blood ammonia levels.

**Conclusion:** The amount of ammonia produced by *H. pylori* does not affect venous ammonia levels. Therefore, an additional benefit of *H. pylori* eradication in the treatment of hepatic encephalopathy in patients with LC is unlikely to occur.

**Key words:** minimal hepatic encephalopathy – subclinical hepatic encephalopathy – CFF – *H. pylori* – liver cirrhosis – NCT-A.

**Abbreviations:** CFF: critical flicker frequency analysis; GCP: good clinical practice; GI: gastrointestinal; HE: hepatic encephalopathy; *H. pylori*: Helicobacter pylori; LC: liver cirrhosis; MHE: minimal hepatic encephalopathy; NASH: non-alcoholic steatohepatitis; NCT-A: number connection test-A; NCT-B: number connection test-B; PHES: Psychometric hepatic encephalopathy score.

**INTRODUCTION**

Minimal hepatic encephalopathy (MHE) is defined as a subclinical brain function disorder due to the worsening of chronic or acute liver parenchyma damage or to portosystemic shunting. Patients with MHE have a significantly restricted daily life, with impaired ability to work and to drive motor vehicles. Minimal hepatic encephalopathy is clinically unapparent; it is diagnosed by alterations of tests exploring psychomotor speed, executive functions or neurophysiological alterations without clinical evidence of mental change [1]. Hepatic encephalopathy (HE) in its various grades, according to the West Haven Criteria, is estimated to be present in 30–45% of persons with liver cirrhosis (LC), while approximately 60–80% of these persons show cognitive dysfunction in specialized testing. All clinical manifestations of HE are characterized by the absence of any structural cerebral changes and are potentially reversible by adequate therapeutic management [2–4].

Only a minority of gastroenterologists are prepared to test for MHE. In a survey conducted in the USA, 38% of the members of the American Association for the Study of Liver Diseases had never tested for it, although the majority (84%) are aware of the medical problem and the need to test for it [5].
The diagnostic pathway of MHE includes neurophysiological and psychometric tests. However diagnostic standards and criteria are not consistent [1] and MHE often remains undiagnosed owing to its minimal to mild symptomatic expression.

Our picture of the pathogenesis and development of HE is still incomplete, but ammonia is considered to be most relevant [7, 8]. Treatments with non-absorbable antibiotics and non-absorbable disaccharides aim at the reduction of bacterial ammonia production in the large bowel and have been proven to be effective against MHE. Apart from ammonia, other factors are likely to be involved in the pathogenesis of HE: mercaptans, aromatic amino acids, short- and medium-chain fatty acids and endogenous benzodiazepines. In patients with LC the detoxification capacity of the liver is impaired, which permits these substances to be present in the systemic circulation in increased amounts and, in the case of molecules able to pass the blood–brain barrier, leads to increased levels in the cerebrospinal fluid. Morphological changes – low-grade cerebral edema – lead to alterations of neurotransmitter receptor systems and changes in synaptic plasticity.

Among bacteria, Helicobacter pylori has also been suggested as possible source of ammonia production because of its high urease content [6, 11–13]. A possible role of H. pylori infection in the pathogenesis of HE has been investigated in several observational studies, and 12 interventional trials have assessed the effect of H. pylori eradication in patients with liver cirrhosis and HE. Most of these studies addressed patients with overt HE. A systematic review [6] summarizes current knowledge regarding a possible association between H. pylori infection and HE; it concluded that there is no clear evidence of a possible positive effect of eradication therapy on hyperammoniemia and HE.

There are only few data published so far from small prospective clinical studies on the role of H. pylori in subclinical HE, and their findings are inconsistent [9, 10].

In this study we investigated the prevalence of H. pylori infection in patients with MHE and the influence of the infection upon serum ammonia concentration in patients with LC, and also the correlation of serum ammonia concentrations with psychometric test results and presence of H. pylori infection.

METHODS

Between January 2013 and December 2014, a total of 84 consecutive cirrhotic patients (70 male, 14 female) cared for in the outpatient ward of the Department of Gastroenterology, Hepatology and Infectious Diseases of Magdeburg University Hospital, Germany, were enrolled. The study protocol was performed in accordance with current Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki and approved by the Ethics Committee of the University of Magdeburg (Committee’s study identification number 177/12). All patients gave their written informed consent to participate.

The epidemiological and clinical data acquired included sex, age, etiology of LC, information 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 important exclusion criteria were antibiotic therapy during the previous four weeks, status after eradication therapy for H. pylori, ongoing therapy for MHE, axis deviation of the eyes, color blindness, inability to take part in the neurophysiological and psychometric tests, and the presence of overt HE.

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

Detection of H. pylori infection

Fasting venous blood samples were taken from each patient to determine anti-H. pylori IgG by using the H. pylori IgG enzyme-linked immunosorbent assay (Biohit, Rosbach, Germany). All samples were analyzed according to the manufacturer’s instructions. The classification of H. pylori status was performed on the basis of the H. pylori-specific IgG (≥ 30 enzyme immunounits counted as positive).

Ammonia measurement

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

Psychometric diagnostic procedures for MHE

Number-connecting test (NCT)

The NCT-A test was performed by a physician or a suitably trained medical student to detect MHE. The patients were required to connect increasing numbers from 1 to 25 printed on a piece of paper, as quickly as possible. A pathological finding was recorded if more than 30 seconds were needed to complete this task.

Critical flicker frequency (CFF) analysis

For the assessment of MHE the HEptonorm™ Analyzer (HE Flicker Diagnostics GbR, Düsseldorf, Germany) was used. Intrafoveal stimulation with a luminous pulsating light-emitting diode requires a precise lens system with unimpaired eye accommodation. A continuously, gradually decreasing frequency of stroboscopic light pulses, starting with 60 Hz and ending with 25 Hz, is stopped by the patients when they have the impression of a flickering light. After a brief instruction and training period, 10 measurements were performed and the mean stopping frequency was calculated; a cut-off value of < 39 Hz was used to detect impairment.

All measurements were performed between 8:00 a.m. and 12:00 a.m. in a quiet room under constant ambient light conditions.

Statistical analysis

A database was created in Excel 2010 (Microsoft Corporation, Redmond, Wash., USA) 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 means with standard deviation. For categorical data, results are given as absolute numbers with percentages. For comparison of categorical data, the χ² test was applied if the expected incidence exceeded...
RESULTS

A total of 84 patients (83% of them male) with LC were screened for MHE and hyperammoniemia and were serologically tested for \textit{H. pylori} infection. None of the patients had a history of \textit{H. pylori} eradication therapy, and none had previous episodes of overt HE. Demographic characteristics are described in Table I.

Of the patient collective, 56% (47) were suffering from alcohol-induced liver cirrhosis, 17% (14) from non-alcoholic steatohepatitis (NASH) cirrhosis and 8% (7) had a history of alcohol abuse and metabolic risk factors for non-alcoholic fatty liver disease. In 17% (14), other causes of LC were diagnosed (Table I). The severity of LC was graduated by using the Child–Pugh score.

Table I. Baseline characteristics of the patient cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>n/mean</th>
<th>% / SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>70</td>
<td>84</td>
</tr>
<tr>
<td>female</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Age, years</td>
<td>61.9</td>
<td>9.0</td>
</tr>
<tr>
<td>Etiology of liver cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alcohol</td>
<td>47</td>
<td>56</td>
</tr>
<tr>
<td>NAFLD</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>HCV</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>HBV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AIH</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>mixed</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>other</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>unknown</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Child–Pugh stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>B</td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td>C</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>unknown</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>\textit{H. pylori} status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>negative</td>
<td>66</td>
<td>79</td>
</tr>
</tbody>
</table>

Blood ammonia levels

Increased venous ammonia levels were detected in 31 cirrhotic patients (37%). Of the patients with MHE diagnosed by CFF, 19% presented with increased ammonia levels, and in patients infected with \textit{H. pylori} 33% showed ammonia levels above the threshold (Tables II–IV).

Results of psychometric and neurophysiological tests

On the basis of CFF, 24 patients (29%) had pathological findings implying a diagnosis of MHE.

NCT-A demonstrated abnormal findings corresponding to MHE in 61 patients (73%). Stratified by Child–Pugh stage, CFF detected MHE in 13 cases in Child A, in 8 cases in Child B and in 2 cases in Child C (for one patient the Child–Pugh stage was unknown), whereas NCT-A revealed pathological findings in 30 cases with Child A, in 20 cases with Child B and in 9 cases with Child C cirrhosis. When a positive MHE result was defined as a positive test result in both NCT-A and CFF, MHE was diagnosed in 23 of the 84 patients (27%).

Eighteen patients were found to be \textit{H. pylori}-positive (21%) and 22% of \textit{H. pylori} infected cases presented with pathological findings in CFF, whereas 17% of infected patients had pathological findings when a positive overall result comprised concordant positive results in both CFF and NCT-A.

There was no significant association between the results of CFF and NH4 (p = 0.153), while the results of CFF and NCT showed a significant association (p = 0.003; Table III).

Table II. Results of psychometric testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>n/mean</th>
<th>%/(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFF</td>
<td>&lt; 39 Hz</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>≥ 39 Hz</td>
<td>60</td>
<td>71</td>
</tr>
<tr>
<td>NCT</td>
<td>&gt; 30 sec</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>≤ 30 sec</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>NH4 level</td>
<td>≤ 55.3 μmol/l</td>
<td>53</td>
<td>(63)</td>
</tr>
<tr>
<td></td>
<td>&gt; 55.3 μmol/l</td>
<td>31</td>
<td>(37)</td>
</tr>
</tbody>
</table>

CFF: critical flicker frequency analysis; NCT: number connection test

Correlation of minimal hepatic encephalopathy with the presence of \textit{H. pylori} infection

The prevalence of MHE did not differ significantly between patients with \textit{H. pylori} infection and those without such infection, irrespective of whether the presence of MHE was defined by CFF alone, by NCT-A alone, or by a combination of CFF and NCT-A (Table IV).

DISCUSSION

\textit{H. pylori}, with its high urease content, has been suggested to be a contributor to intestinal ammonia production. Ammonia is an essential factor linked to the pathogenesis of HE. Observational and interventional studies on the association of \textit{H. pylori} infection with HE and on the reduction of ammonia by \textit{H. pylori} eradication have not been conclusive [6]. Results on the association of \textit{H. pylori} infection with MHE in cirrhotic patients are limited. We present the findings of the analysis of the largest yet cohort of patients with MHE and the relationship with \textit{H. pylori} infection, in the context of the fact that MHE is defined as a subclinical manifestation of HE without a clearly defined gold standard for its diagnosis.
In our study the serological \textit{H. pylori} prevalence in the LC patients tested (21\%) is low compared with that in an unselected cohort of patients from our region (45\% as published previously) [14]. \textit{H. pylori}-positive patients showed normal ammonia levels in 67\% of cases as compared with 62\% in \textit{H. pylori}-negative patients with LC. This indicates that \textit{H. pylori} infection cannot be regarded as a principal source of ammonia production [15].

These findings confirm the results of previous studies on the role of \textit{H. pylori} infection in subclinical HE that showed no significant effect of the infection on cognitive function in patients with LC [9, 10, 16–18]. They stand in contrast to the findings of smaller studies, which revealed a high prevalence of serologically detected \textit{H. pylori} infection in cirrhotic patients with overt and occult HE and implied a positive correlation between the severity of HE and the level of \textit{H. pylori} antibodies, i.e., above or below cutoff [19, 20].

The reason for this discrepancy with results from previous studies is to be found in the lack of a gold standard for the diagnosis of MHE. Recent studies have favored the use of the psychometric hepatic encephalopathy score (PHES), a combination of five different psychometric tests (NCT-A, NCT-B, serial-dotting test, line-tracing test and digit-symbol test). In clinical routine, tests have to be time-saving, reliable and reproducible. CFF alone distinguishes between patients with overt HE and those without minimal or overt HE with a 98\% sensitivity and 94\% specificity. For the detection of MHE the test is highly specific, but has a sensitivity of only 37\% [21]. However, neither PHES nor a combination of PHES with CFF is able to distinguish reliably between the presence and absence of MHE, and the tests recommended by current guidelines correlate poorly because HE is a multi-dimensional dysfunction [1]. We therefore decided to use CFF to define the presence of MHE but also, additionally, to use the NCT-A and a combination of both tests in the analysis. However, our results also demonstrate only a weak correlation between NCT-A and CFF.

Our results further demonstrate that there is no added benefit of measuring blood ammonia levels in LC patients without overt HE to detect MHE. Previous studies have shown that systemic levels of ammonia in MHE do not correlate with the severity of liver disease and the presence of MHE [22, 23]. Contrary to that, there is a positive correlation of ammonia blood levels with the stage of HE in overt HE [24]. Ammonia measurement is therefore useless as a screening test for MHE.

The relative small number of patients and the use of serology for the diagnosis of \textit{H. pylori} infection are the limitations of our study. A prolonged decrease of anti-\textit{H. pylori}-IgG antibodies after (accidental) eradication may reduce the specificity. We excluded patients with previous antibiotic therapies and status after eradication therapy for \textit{H. pylori} to eliminate this confounding factor.

CONCLUSION

The amount of ammonia produced by \textit{H. pylori} does not influence ammonia serum levels and is not associated with an increased prevalence of MHE in cirrhotic patients. An additional benefit of \textit{H. pylori} eradication in the treatment of HE in patients with LC cannot be expected. However, apart from the prevention of gastroduodenal complications, a potential positive effect of \textit{H. pylori} eradication therapy on the cognitive function in patients with LC through the modulation of the intestinal microbiome further down in the GI tract could be a worthwhile object of future evaluation.

Conflicts of interest: The authors have no competing interests.

Authors’ contribution: C.S.: study concept and design, data acquisition, analysis and interpretation; manuscript drafting. K.S.: data acquisition, analysis and interpretation, manuscript drafting, critical revision of the manuscript for important intellectual content; N.R. and J.V.: data acquisition, collection and analysis (this study is the basis of their medical theses); P.M.: study supervision, critical revision of the manuscript for important intellectual content.

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