Association Of Electroencephalogram Patterns With Ammonia Levels In Hepatic Encephalopathy Patients

Anam Fatima¹, Faridullah Shah², Hareema Saeed Khan³, Kashif Rauf⁴, Asif Jalil⁵, Muhammad Saleem Akhter⁶

Abstract

Objective: To find out the association between electroencephalogram (EEG) and hepatic encephalopathy.

Methodology: This cross-sectional study included 100 patients (with the age of 52.5 ± 6.09 years for males and 51.7 ± 6.10 years for females) of reported hepatic encephalopathy, visiting the medical department (indoor and OPD) at Federal Government Polyclinic Hospital, Islamabad. The study was conducted from January 2020 to May 2021. Patients who had known epileptic and structural brain lesions or strokes were excluded from the study. Statistical analysis was done using GraphPad Prism software. The significance of data (p-value or R² value) was calculated through a two-tailed test or correlation coefficient.

Results: All the patients in hepatic encephalopathy grade IV reported abnormal EEG representing triphasic waves and flattening of EEG pattern. There was no correlation observed between age, gender and hepatic encephalopathy grades. However, a significant correlation (R² = 0.9032) was observed between serum ammonia levels and hepatic encephalopathy grades. Elevated serum ammonia levels depicted the severity of hepatic encephalopathy. Overall, the percentage of patients with abnormal EEG increased with increasing grade of hepatic encephalopathy. It was quite intriguing to note that EEG, being the common method to diagnose hepatic encephalopathy grades, is not dependent on patients’ socio-economic status.

Conclusion: Data concluded that serum ammonia levels are well associated with the progression of hepatic encephalopathy. Moreover, the EEG pattern provides the appropriate information about the neurological abnormalities associated with the severity of hepatic encephalopathy. Hence, serum ammonia levels and EEG both should accurately be used as indicators for diagnosis and monitoring the response to the treatment of various grades of hepatic encephalopathy. Data warrant further investigations to get a better insight into hepatic encephalopathy’s relationship with EEG patterns through the inclusion of molecular parameters.

Keywords: Hepatic Encephalopathy; Electroencephalogram; Triphasic Wave; Cirrhosis; Ammonia.

1. Introduction

Liver dysfunction called hepatic encephalopathy (HE) increases blood toxins which translates into many neuropsychiatric complications in the brain. It causes abnormalities in sleeping behaviours, moods leading to coma (1-3). A variety of diagnostic tools and protocols for HE, including patients’ neurological and psychiatric evaluation and electroencephalography (EEG), are being used in clinics (4-6). HE is having a complication of cirrhosis that may lead to parkinsonism. The pathogenesis of HE is associated with many underlying mechanisms including toxins accumulation in the blood which circulate to the brain. Ammonia being a neurotoxin affects brain function. Pro-inflammation, auto-immunity to brain antigens (7) and abnormalities in neuro-transmission mechanisms are also associated with HE pathogenesis (8, 9). Lowering the neurotoxin levels in serum improves HE conditions. This can be achieved through the intake of antibiotics, lactulose or the usage of non-coding RNA molecules (9-11).

EEG patterns identify the HE through abnormal behaviour of waves produced in the presence of brain dysfunction as compared to merely liver disease in the absence of brain dysfunction. This EEG pattern is known as triphasic waves (3, 12).

The earliest detectable changes in HE patients which describe brain dysfunction through slow and disorganized patterns is called alpha rhythm (6). Progressively, the alpha rhythm is converted into another frequency phase which is characterized at 4-7/ second in the cortex of the brain and is called theta phase. During theta phase, the patient feels mild disorientation regarding the timescale and place. Further clinical complication increases the
disorientation in patients and EEG patterns change to slow triphasic waves. Patients in the triphasic phase get into a coma and arousable only through painful stimuli. During this severe complication, patients present the following different EEG patterns: firstly) diffused wave pattern (theta stage) in EEG showing a frequency of 4-7/sec, secondly) synchronized and diffused bilateral triphasic EEG patterns can be seen with a maximum positive deflection at the surface, and thirdly) a random, mild bilaterally synchronized EEG pattern called delta phase can be seen as a dominant pattern (13, 14). Previous studies reported that control groups have depicted normal EEG patterns while patients in theta or triphasic phases have been shown as confused or unresponsive coma in theta and triphasic phases, respectively (12, 15).

Previous studies reported multiple reasons for EEG patterns related to coma or stupor. Among these causes include renal or metabolic dysfunctions. EEG patterns in these dysfunctions are associated with slow rhythms and triphasic wave patterns. Lesser supply of oxygen and the presence of toxins in the brain also aggravate the conditions in HE patients which can be seen as abnormal EEG patterns (16). Restricted blood supply to the brain tissues also causes the onset of triphasic wave patterns in HE patients. (17, 18)

EEG patterns permit the analysis of thalamocortical function in patients with clinical coma conditions. Continuous monitoring of the coma condition through EEG patterns helps to assess the efficacy of the drugs administered to HE patients (19, 20).

Previous studies showed a lacking EEG association with various grades of HE. It would be the logical basis to investigate the association further to develop a more specific diagnostic point which would help for better treatment of patients with HE. Hence, the current study aimed to understand the association between EEG and HE by measuring serum ammonia levels.

2. Materials & Methods

In this cross-sectional study, a cohort (n=100) comprised of both male and female patients was included with reported HE visiting the medical department (indoor and OPD) of Federal Government Polyclinic Hospital (FGPH), Islamabad. Data from all patients were collected from January 2020 to May-2021. The study was approved by the Bio-Ethical Committee of FGPH, Islamabad (FGPC.1/12/2021/Ethical Committee).

Patients who are known epileptic and with structural brain lesions or stroke were excluded from the study. Moreover, children under the age 12 years were also excluded from the study as they don’t develop HE clinically. A previous study reported that the average age of HE development is over 50 years. (21)

All the participants were explained and informed (consent was taken) about the purpose of the study, methodology and usage of the collected information and data. Confidentiality of data and information collected were also ensured to the participants. The sample size was collected as per randomized sampling concept by keeping confidence level at 95%. With n=100, margin of error was calculated as ±9.60%.

Demographics, gender, age and socio-economic status of the patients was collected by a questionnaire which is a common method to collect data for cross sectional studies. (22, 23) The questionnaire was presented by the staff to the patients. Serum ammonia (NH3) levels and electroencephalograms (EEG) were collected from the patients’ lab and neurologists’ reports, respectively.

Serum ammonia levels were measured as described by the manufacturer (Abcam, UK) and were commonly used in previous studies. (24-26) Briefly, fresh blood samples were collected in serum tubes (BD, USA). Serum was collected and transferred into a clean tube. All procedures were performed on ice. Reagents were prepared immediately before the assay. The ammonia reaction mix and background mix were prepared and loaded into the 96-well plate. Sample master mix, control and standard mixtures were loaded in duplicate. Microplate was incubated at 37°C for 1h. Microplate was read on a microplate reader (BioTek, USA) at OD 570nm.

EEG pattern was recorded as per manufacturer’s protocol by using the machine Natus Neurology Nicolet EEG V32 (USA). Head cap having electrodes was placed on patients’ head, eyes were closed (to minimize artifacts due to eye blinking) and electroencephalogram pattern was recorded by EEG recorder and graph was made by EEG reader having Nicolet One Modular Neuro-diagnostic Software SystemVersion 5.94.

Clinical grading of HE patients was done and their serum ammonia levels were recorded. EEG of these patients was done and patterns of wave forms in different stages of HE were studied.

Statistical data analysis
All the data were analyzed through GraphPad PRISM v.5.0 software. The correlation coefficient (best-fit curve) was used to study the variables. Data were calculated as ±SD. Correlation coefficient R²=0.9 is considered as significant or positive correlation. The p value was calculated by two-tailed test for the Figure 2a and b. The p value ≤ 0.05 is considered as significant.

3. Results

Study cohort

Study cohort (n=100) comprised of both male (53%) and female (47%) patients visiting indoor and OPD of the medical department at Federal Government Polyclinic Hospital (FGPH), Islamabad. Average age was 52.5 ± 6.09 years for males and 51.7 ± 6.10 years for female. Ammonia (NH3) concentrations were recorded as 60±30.51 and 61±30.57 μmol/L of males and females, respectively.

Table 1: EEG pattern corresponding to HE grades in total patients (n=100)

<table>
<thead>
<tr>
<th>Hepatic Encephalopathy Grade</th>
<th>No. of Patients</th>
<th>Electroencephalogram Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>I</td>
<td>30(30%)</td>
<td>27(90%)</td>
</tr>
<tr>
<td>II</td>
<td>35(35%)</td>
<td>20(57.14%)</td>
</tr>
<tr>
<td>III</td>
<td>30(30%)</td>
<td>5(16.67%)</td>
</tr>
<tr>
<td>IV</td>
<td>5(5%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Changes in EEG pattern during HE

Out of 100 patients, 30%, 35%, 30% and 5% patients were reported for hepatic encephalopathy (HE) grades I, II, III and IV, respectively. All patients of HE grade IV represented abnormal (100%) EEG pattern. However, 10%, 42.86% and 83.33% patients showed abnormal EEG from HE grade I, II and III patients’ pool, respectively (Table 1). Moreover, 90%, 57.14% and 16.67% patients showed normal EEG patterns having HE grade I, II and III, respectively. There was not any patient with normal EEG pattern having HE grade IV. Patients with HE grade I (10%) and II (42.86%) reported slow waves and theta rhythm of the EEG pattern. It was intriguing to note low amplitudes low waves among 20% patients, slow waves and theta rhythm among 32% patients and a few triphasic waves along with mild theta and delta EEG pattern among 48% patients of HE grade III. Moreover, HE grades IV patients (80%) depicted flattening of EEG while the remaining 20% patients depicted triphasic waves with delta rhythm (Table 2).

Table 2: Distribution of abnormal EEG pattern

<table>
<thead>
<tr>
<th>Hepatic Encephalopathy Grade</th>
<th>No. of Patients with Abnormal EEG</th>
<th>EEG Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3(10%)</td>
<td>Slow waves</td>
</tr>
<tr>
<td>I</td>
<td>15(42.86%)</td>
<td>Slow waves, theta rhythm</td>
</tr>
<tr>
<td>I</td>
<td>5(20%)</td>
<td>Low amplitude and slow waves</td>
</tr>
<tr>
<td>I</td>
<td>8(32%)</td>
<td>Slow waves, theta rhythm</td>
</tr>
<tr>
<td>IV</td>
<td>12(48%)</td>
<td>Triphasic wave, delta rhythm</td>
</tr>
<tr>
<td>IV</td>
<td>1(20%)</td>
<td>Triphasic wave, delta rhythm</td>
</tr>
<tr>
<td>IV</td>
<td>4(80%)</td>
<td>Flattening of EEG</td>
</tr>
</tbody>
</table>

Changes in EEG pattern during HE

EEG pattern in HE grade I depicted low-frequency waves. Random theta rhythm was observed in HE grade II along with low amplitude and slow waves. Most of the patients in the HE grade III group depicted theta rhythm with mild delta rhythm. However, triphasic EEG pattern was also observed here. HE grade IV patients showed predominant flattening of EEG pattern along with triphasic waves (Figure 1).
Correlation between HE grades and serum NH₃ levels

A significant ($R^2=0.9032$) correlation was observed between serum ammonia (NH₃) levels and HE grades. Serum NH₃ levels gradually increased along the four types of HE grades. However, data showed that HE grade I occasionally depicted close to normal serum NH₃ levels, while these levels changed to abnormal levels for other HE grades II-IV (Figure 2b).

Distribution of serum NH₃ levels

Averages serum NH₃ levels ($\mu$mol/L) were found almost equal in male and female patients irrespective of their HE grades (Figure 2c).

5. Discussion

Data showed various HE grades among the patients included in the study. Results showed that males and females were equally prone to develop any stage of HE. Moreover, there was no correlation observed between gender or age with various grades of HE and the data are in line with a study conducted at European cohort having HE. (27) However, a study conducted by Helzberg and colleagues in 2021 reported that a low socio-economic status was prone to develop HE. (28)

Data depicted that nearly equal number of patients were in I-III stages of HE and less number was reported in grade IV. It is justified, because most of the patients usually presented to the hospital for treatment in early stages and they are treated before progression of the disease to HE grade IV. Previous studies depicted that acute HE is reversible through various liver treatment or transplant procedures (29, 30) while chronic or grade IV HE is hard to recover except liver transplantation. (31)

Out of 30 patients in HE grade I, 90% patients were had normal patterns of EEG while the rest 10% patients depicted slow waves. EEG reflects postsynaptic activity of cortex region of the brain which is highly sensitive to toxins and other metabolic factors. (32) EEG is
preferably a vital diagnostic procedure for HE detection and monitoring. (31)

Rhythmic background activity and transients are the two vital parts of EEG patterns for HE patients. Progression of EEG patterns from slowing the rhythms to the development of the triphasic stage is corresponding to the shift from mild to severe HE (6, 33) This pattern was observed in our data where HE grade I showed slow waves and theta rhythm and subsequently it shifted to triphasic waves followed by flattening of the waves in HE grade II and IV, respectively. Correspondingly, the percentage of the patients was also shifted towards the highest number from grade I to grade IV HE.

Initially, EEG is described by an increase in cortical beta activity which decreases in more severe hepatic encephalopathy and there are runs of theta rhythm. (34) This EEG pattern is also reflected in our data where abnormal EEG was observed progressive along the HE grades. It was quite intriguing to note that EEG rhythms progressively getting abnormal from delta to flattening of the pattern. Flattening of the EEG reflects the irreversible loss of consciousness. (35)

Slowing wave frequencies in EEG patterns during HE are not un-common. Onset of encephalopathy is associated with changes in the patterns of rhythms which progressively worsen in complex conditions. Theta waves progressively shifted to delta stage which is corresponding to the development of coma in HE patients and indicate the severity of encephalopathy. With further increase in the severity of coma, EEG becomes flat. This stage is called electro-cerebral inactivity or silence. (35)

Elevated serum ammonia levels reflect the worsening of encephalopathy as depicted by our data and also reported by previous studies. (12, 36) Additionally, EEG pattern is also linked with elevated serum ammonia concentrations. In patients with high grade encephalopathy and elevated serum ammonia toxicity, a typical triphasic pattern of EEG can be easily observed. (37)

5. Conclusion

Data concluded that serum ammonia levels are well associated with progression of HE. Moreover, the EEG pattern provides the appropriate information about the neurological abnormalities associated with severity of HE. Hence, EEG and serum ammonia levels both should accurately be used as indicators for diagnosis and monitoring the response to the treatment of various grades of HE. Data warrant further investigations to get a better insight into HE relationship with EEG patterns through inclusion of molecular parameters.

CONFLICTS OF INTEREST- None

Financial support: None to report.
Potential competing interests: None to report
Contributions:
A.F, F.S - Conception of study
A.F, F.S - Experimentation/Study Conduction
A.F, F.S, H.S.K, K.R - Manuscript Writing

References


