Ammonia levels in patients with generalised epilepsy that are on sodium valproate monotherapy.

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, for the degree of Master of Medicine in the division of Neurology.

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I. DECLARATION

I, Kareli Jonker, hereby declare that the research report is my own work. It is being submitted for the Degree of Master of Medicine in the division of Neurology, University of Witwatersrand, Johannesburg. This research report is submitted in the published format as recognized by the Faculty of Health Sciences. I further declare that this work has not been submitted for any other examination or degree at this or any other University.

Dr.Kareli Jonker

Student number: 2419928

16th day of October 2025 in Johannesburg

II. ETHICAL CONSIDERATIONS

Permission for this cross-sectional study was obtained from each hospital at which this study was conducted (Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital) as well as from the Human Research Ethics Committee (HREC) of the University of Witwatersrand (clearance number: M220701).

III. ABSTRACT

Background: Epilepsy is a prevalent neurological disease frequently managed in outpatient clinics at tertiary hospitals. Sodium valproate is widely utilized within the public healthcare sector in South Africa for epilepsy management due to its affordability and accessibility, despite its limitations, particularly in patients of childbearing age. Extensive research published in international literature has demonstrated that sodium valproate can induce hyperammonemia, which may contribute to cognitive dysfunction and a higher frequency of seizures. This study aimed to evaluate the proportion of patients on sodium valproate monotherapy who display elevated serum ammonia levels.

Methods: Patients were interviewed at neurology clinics at Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital during their follow-up visits between December 2021 and December 2022. They were not recruited based on new symptoms. Blood samples were collected to measure ammonia levels, sodium valproate levels, and liver function tests. A data collection sheet was completed, detailing the dose of sodium valproate, duration of treatment, chronic medication used, changes in seizure frequency, changes in cognition, and neurological examination since the initiation of the drug. Descriptive statistics were performed for all variables, and the cohort was further categorized into primary and secondary epilepsy.

Results: This cross-sectional study evaluated 64 adult epileptic patients on sodium valproate (VPA) monotherapy. After excluding twenty-seven patients, the final cohort consisted of 37 patients. Elevated ammonia levels were observed in 30% of the entire cohort (n=37), with a higher prevalence of 40% among those with primary generalized epilepsy (n=20). The reference range for normal serum ammonia levels in this study was 11-35 µmol/L, with elevated levels defined as those exceeding 35 µmol/L. Among the patients with raised ammonia levels (n=11), one exhibited altered cognition and the other had a higher frequency of seizures. The majority was asymptomatic.

Conclusion: From this research study, it can be concluded that 30%-40% of the outpatients on VPA monotherapy had raised ammonia levels and most of them were asymptomatic.

IV. ACKNOWLEDGEMENTS

I am grateful to Dr. Kalpesh Jivan for his insight and guidance as my supervisor.
I extend my sincere gratitude to Dr. June Fabian for her invaluable expertise and knowledge in the field of research.
To Alick Jonker for his assistance with the statistical analysis.

V. LETTER OF CONTRIBUTION

The principal investigator recruited the majority of the patients, with assistance from four other neurology registrars who were responsible for collecting data and blood samples.

Contents

NOMENCLATURE

ARV: Antiretroviral drug

ASM: Anti-seizure medication

EEG: Electroencephalography

GGT: Gamma-Glutamyl Transferase

ILAE: International League against Epilepsy

LFT: Liver function tests

NHLS: National Health Laboratory Services

NICE: National Institute for Health and Care Excellence

PGE: Primary generalized epilepsy

TB: Tuberculosis

VIE: Valproate induced encephalopathy

VIH: Valproate induced hyperammonemia

VPA: Valproic acid/ Sodium valproate

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1. LITERATURE REVIEW

1.1.Background

1.1.1. General information on epilepsy

Epilepsy is a common neurological condition in which recurring, unprovoked seizures occur due to abnormal electrical discharges in the brain. The estimated incidence is 33 to 57 per 100 000 people worldwide, accounting for 1% of the global burden of disease (1). A seizure can be defined as increased discharges of cortical neurons and epilepsy defined as a propensity to have seizures (2). The International League against Epilepsy (ILAE) revised classification of 2017 classifies seizures as focal, generalized and unknown (3).

Generalized seizures originate in both hemispheres and can further be divided into non-motor e.g. absence, and motor e.g. myoclonic, atonic and tonic-clonic seizures. Unknown seizures cannot be categorized as focal or generalized due to a lack of clinical information, EEG and video monitoring available to distinguish between the two types (3). The aetiology of seizures includes genetic abnormalities, structural abnormalities, CNS infections, immune mediated conditions, metabolic derangements, and lastly, idiopathic (3). The diagnosis of generalized seizures is based on clinical grounds and EEG changes. The EEG typically reveals generalized inter ictal spike and wave discharges but can also be normal (4).

1.1.2. Sodium valproate use in epilepsy

Most patients with epilepsy are treated with a single anti-seizure medication (ASM) and current guidelines from the National Institute for Health and Care Excellence (NICE) recommend lamotrigine, levetiracetam or sodium valproate (VPA) as first-line treatment for generalized onset seizures in adults (5). When using sodium valproate, one should adhere to the Medicines and Healthcare Products Regulatory Agency (MHRA) safety measures. These measures warn against the use of VPA in patients of childbearing age. It is typically not recommended for patients under 55 years old unless two specialists independently determine and document that no other effective or tolerated treatment options are available, or there are compelling reasons indicating that reproductive risks do not apply (5).

VPA is commonly used in the state sector of South Africa due to its affordability and availability when compared to the other anti-seizure medication (6).

ASM's suppress seizure activity by reducing neuronal excitability and thereby reducing the probability of seizures occurring. Sodium valproate potentiates gamma-aminobutyric acid (GABA) and blocks voltage gated sodium channels during neuronal excitability (7). Figure 11.1, Appendix 1. The appropriate choice of an ASM is the key to therapeutic success, given that about 70% of patients with epilepsy become seizure free after being treated with one ASM (1).

1.1.3. Sodium valproate levels and seizure control

The ASM concentration varies from one patient to another, and the variability of the VPA level is dependent on drug absorption, metabolism and drug interactions (7). Table 11.1, appendix 2. Sodium valproate is 90% plasma protein bound, and the degree of binding decreases as the drug dosage increases. This results in a nonlinear relationship between total plasma concentration and dosage (7). The correlation between plasma drug concentrations and clinical response has been investigated in many studies and has been found to be poor (7,8). The initial goal should be to achieve the plasma concentrations within the reference range until clinical response to therapy can be assessed (8). It is important to treat the patient according to their seizure frequency rather than just relying on their ASM blood concentration (8). The risk of seizures was found to increased 2.65- fold for every 100umol/L rise in serum ammonia (9). Raised ammonia levels have been shown to impair the potassium-buffering capacity of astrocytes, thereby contributing to increased neuronal excitability and a lower seizure threshold (10).

1.1.4. Sodium valproate and liver function tests

Sodium valproate can derange liver functions by its enzyme inducing potential. The highest incidence of hepatotoxicity is observed when co-administering cytochrome P450 (CYP) enzyme inducers such as phenobarbital and phenytoin (11). Patients below the age of two and, those with pre-existing liver failure and mitochondrial disease have an increased susceptibility to liver dysfunction, but for the rest of the population without these risk factors, the incidence of liver dysfunction is less than 0.02% (11). Aminotransferase levels are only mildly elevated in most patients (11). In rare cases these values exceed 25-fold elevations (1000 IU) (12). Values for aspartate aminotransferase (AST) are approximately equal to those for alanine aminotransferase (ALT). Severe hepatotoxicity correlates poorly with the VPA and serum aminotransferase levels (11). Monitoring of liver function test (LFT) should be done during the first 6 months to detect early hepatotoxicity (11). Liver dysfunction in general can

manifest clinically with apathy, increased somnolence, anorexia, vomiting and increased seizure frequency. Management of liver dysfunction involves discontinuing the drug after excluding other potential causes of liver dysfunction. Treatment options include administering lactulose to reduce ammonia levels, providing L-carnitine supplementation, and referring the patient to a specialist for haemodialysis (13,14).

1.1.5. Sodium valproate and hyperammonemia

Valproate induced hyperammonemia (VIH) is defined as raised serum ammonia levels in patients with normal liver function tests, who are on sodium valproate (15). The ammonia level is considered raised if it is above the upper limit of the normal range, which typically in adults is 50 µmol/L (may vary slightly between laboratories) (16). The incidence of hyperammonemia has been reported to be 0%–56% in patients receiving VPA monotherapy. The high variability in incidence may be related to the different definitions and cut-off values for hyperammonemia (12).

Ammonia is broken down to urea by the liver and excreted by the kidneys. One mechanism of VIH is based on the accumulation of toxic metabolites of valproate that inhibits the mitochondrial enzyme, carbamoyl phosphate synthetase 1, thus preventing the completion of the urea cycle (11). This mitochondrial enzyme is needed to convert ammonia and bicarbonate into carbamoyl phosphate (11,17). The second proposed mechanism of VIH is that VPA decreases serum carnitine levels, which is an essential co-factor for the elimination of ammonia in the urea cycle (11,18). Figure 11.2, Appendix 3.

In a review of 24 prospective studies done between 1980 and 2005 it was reported that the prevalence of VIH ranged between 70% and 100%. In most of these studies over 50% of patients were asymptomatic. In those who presented with features of encephalopathy, the raised serum ammonia levels did not correlate with the severity of symptoms and liver functions were all within the normal range (19). Most patients with hyperammonemia are asymptomatic but can present with lethargy, confusion, agitation and increased seizure frequency (15,20). When serum ammonia levels are elevated, glutamate levels in the brain can increase, resulting in astrocyte swelling and cerebral oedema which can lead to increased seizure frequency (21).

1.1.6. Comparative studies

Sodium valproate-induced hyperammonemia is predominantly discussed in international literature. In the absence of South African studies, I focused mainly on three studies conducted in Turkey, Taiwan, and China. These studies present conflicting evidence regarding the correlation between clinical presentation, the use of concomitant antiepileptic drugs, and sodium valproate dosage in patients with elevated ammonia levels during VPA treatment. This will be discussed in the paragraphs below (12,21,22).

In 2007 a study was done by Gomcelli *et al* to document the clinical manifestations of hyperammonemic encephalopathy. This included seven epileptic patients on sodium valproate, of which four were on monotherapy. The duration of therapy ranged from three days to 37 months and doses ranged from 500-1500mg per day. Results revealed that the serum VPA levels were in the therapeutic range and liver function tests were normal. This study also found no correlation between the serum concentration of VPA, the dosage of VPA, serum ammonia levels or severity of VIH. In all their seven patients, the serum ammonia levels were higher than their reference range of 26-46umol/L. EEG monitoring revealed diffuse, symmetric, non-rhythmical slowing in the theta and/or delta ranges in each patient with only one patient's EEG showing epileptiform activity in addition to theta slowing (21).

A prospective, observational study documenting an association between VPA and hyperammonemia in epileptic patients was done by Tseng *et al*. from June 2012 to May 2013, and included 158 patients. Patients on monotherapy or combination therapy were enrolled. The results revealed that the serum ammonia levels correlated with the dosage of VPA and the serum VPA levels. This study also demonstrated that concomitant use of VPA with liver enzyme-inducing ASMs, such as phenytoin, carbamazepine, and phenobarbital, was associated with a higher incidence of hyperammonemia compared to VPA monotherapy (12).

Twenty cases of VIH were identified by Cheng *et al*. between 2000 and 2012. The VPA levels of all patients were within the normal range for their laboratory and their liver enzymes were not raised; however, the mean ammonia level was markedly raised to 138 ± 68 umol/L (14.7-53.3umol/l). Fourteen patients had an abnormal EEG that showed generalized slowing. Most patients (n=14) were on monotherapy. No correlations were observed between serum ammonia levels and serum VPA levels, daily VPA dosage, duration of treatment, polytherapy, age or gender. Most of their patients developed VIH within two months of starting the treatment (22).

These studies did not investigate the change in seizure frequency associated with raised ammonia levels. Clinically, patients were identified as having valproate induced encephalopathy (VIE) based on their level of awareness, their neurological fallout and occurrence of seizures (22). This will be an additional area of investigation as mentioned in the secondary objectives.

1.1.7. Electroencephalogram (EEG) findings in primary generalized epilepsy and sodium valproate induced hyperammonemia

An EEG records the electrical activity occurring at the surface of the brain by using scalp electrodes (23). Patients with PGE typically have findings with normal background activity and ictal sharp and wave activity specific to the seizure type (24).

A cross-sectional study involving the analysis of EEGs of patients with generalized epilepsy, from three centres over a 7-year period, showed that about 58% of patients had an abnormal EEG (4). Generalized sharp and wave complexes were the most common findings among patients with epileptiform activity. Age, poor seizure control and high frequency of seizures were independent predictors of the presence of EEG abnormalities (4).

Electroencephalogram changes due to VIH includes diffuse high-amplitude delta waves or bilateral triphasic waves (25).

2. AIM

This study aims to assess ammonia levels in neurology patients on sodium valproate monotherapy at state hospitals in Johannesburg.

3. STUDY OBJECTIVES

3.1. Primary objective:

To determine the proportion of patients on sodium valproate monotherapy who have raised ammonia levels.

3.2. Secondary objective:

To document the following results in these patients:

- LFT (transaminases and GGT)
- VPA dosage

- Serum VPA level
- Clinical presentation- included documenting any recent changes in cognition, the onset of nausea and/or vomiting, new neurological deficits, and alterations in seizure frequency. Family members or caregivers were queried about any observed changes since the initiation of VPA therapy.

4. METHODS

This multicentre study focused on epileptic patients attending outpatient neurology clinics in Johannesburg. Patients were recruited during their follow-up visits if they were on VPA monotherapy. Consent for the study was obtained during a single encounter, during which blood samples were drawn, a neurological examination was conducted, and a data collection sheet was completed. The characteristics of the population were described based on demographics, collected data, and clinical findings.

4.1.Study design

A cross-sectional and descriptive study with data collection spanning over a year, from December 2021 to December 2022.

4.2.Study setting

Epileptic patients presenting to the neurology outpatient department at Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital, on sodium valproate monotherapy.

4.3.Methods of assessment

A data collection sheet (Appendix 5) was used to gather data from each patient. Patients had already been assessed as having primary or secondary epilepsy and were initiated on sodium valproate during previous clinic visits. The data collection sheet included information on age, sex, self-reported ethnicity, classification of epilepsy (primary versus secondary), duration on sodium valproate (in years), dose of sodium valproate (milligram per day), chronic medications used, and clinical presentation. The clinical presentation section covered any changes in cognition noticed by family members or caretakers, nausea or vomiting, changes in neurological examination since starting sodium valproate, and seizure frequency per month. Patients were also asked if their seizure frequency had decreased or increased since starting sodium valproate. Blood results were recorded once released by the NHLS, including sodium valproate levels, liver function tests (transaminases and GGT), and ammonia levels on ice. Most blood results were available within three days.

4.4.Study population

Inclusion criteria:

- Adult patients with generalized epilepsy on VPA monotherapy.

Exclusion criteria:

- Patients below the age of 16 who follow up at the paediatric outpatient department.
- Pregnant patients.
- Patients on antiepileptic drugs other than VPA.
- Patients with sodium valproate levels <19umol/L – this corresponds to the minimum level measurable by the NHLS.
- Patients who have abnormal transaminases and/or GGT on their LFTs.

4.5.Sampling method

Blood samples were collected in the epilepsy outpatient department and sent to the National Health Laboratory Services (NHLS) for analysis of sodium valproate levels, liver function tests, and ammonia levels. Two tubes were used for collection, one yellow and one purple (EDTA), each containing two millilitres of blood. The samples were delivered to the laboratory within one hour of collection, transported in a cooler box with ice. All specimens were physically delivered to the laboratory by the principal investigator. It was crucial to deliver the specimens promptly and correctly on ice to prevent rejection by the laboratory.

Sample size:

A statistical power analysis was conducted to determine the required sample size for a one-sample mean test. With a population mean of 62.5 and a desired statistical power of 0.8, the calculated minimum sample size was 52 participants. This calculation was performed in consultation with a biostatistician.

Sample selection:

Patients presenting between December 2021 and December 2022 were sampled.

5. STATISTICAL ANALYSIS

Data was analysed using IBM SPSS. The data was coded and matched according to the data sheets. Descriptive analysis of all study variables was presented using frequency and percentage tabulation for categorical variables. For continuous variables, the mean (with standard deviation) or median (with interquartile range) was used, depending on the data

distribution. The proportion of epileptic patients on VPA monotherapy with elevated serum ammonia levels was calculated as a percentage. The cohort was divided in groups based on primary epilepsy versus secondary epilepsy, and elevated ammonia levels.

6. ETHICS

The study was commenced after permission was granted from the Human Research Ethical Committee of the University of the Witwatersrand. (Clearance number: M220701). The information obtained from the medical records was treated with strict confidentiality and was used only for the purpose of the study. Consent was obtained from each patient used in the study. Patient information sheets and consent forms are attached in appendix 6 and 7.

7. RESULTS

7.1. Overall patient sample

Sixty-four (64) patients' information was captured using a data collection sheet. Twenty-seven patients were excluded which included ten patients with VPA levels less than 19umol/L, nine due to lab errors regarding ammonia, five due to abnormal liver function tests, and three had no liver function tests done. Therefore, the sample of the data set used for analysis was made up of 37 patients. Of the 37 patients, 11 had raised ammonia levels.

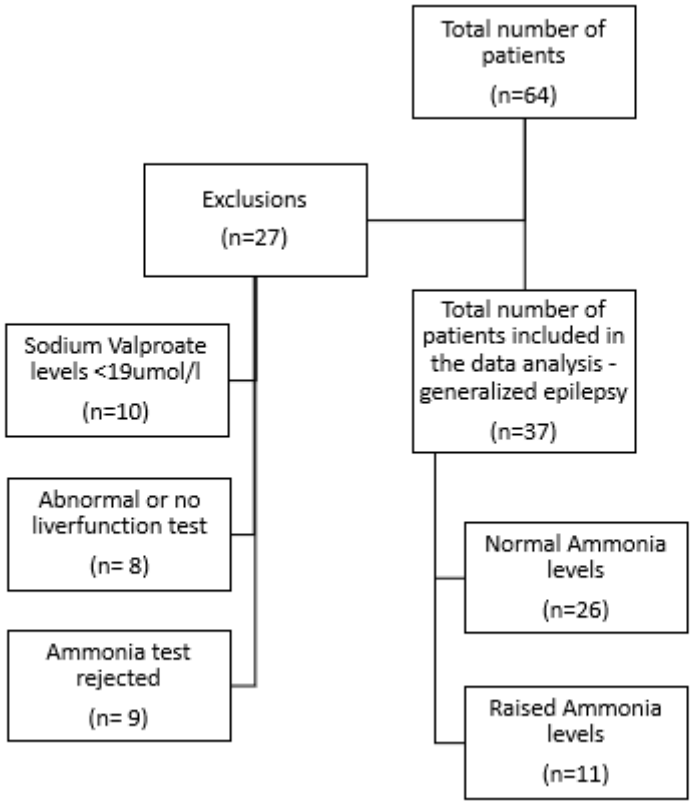


Figure 7.1: Flow diagram of the entire study sample (n=37)

Patients who were not taking their medication could not be included in the study, as the absence of the drug would not affect the ammonia levels. The laboratory rejected nine tests because the samples were not kept on ice, even if they were initially taken on ice, and due to delays in processing, which resulted in unreliable results. Liver function tests were rejected due to insufficient blood samples. Of the five patients with abnormal liver function tests (LFTs), one was undergoing tuberculosis treatment, and another had a significant history of alcohol use. Abnormal LFTs could not be included due to their potential impact on ammonia levels and their role in causing encephalopathy (26).

Most patients in the cohort were young African males on minimal other medications, and the mean seizures per month was less than one. They were further described as having primary or secondary epilepsy based on the information provided as per the data collection sheet. (Table 7.1). Primary epilepsy is defined as seizures that occur without an identifiable structural or metabolic cause. Secondary epilepsy can be caused by tuberculomas, prior infarcts, neurocysticercosis, traumatic brain injury and calcifications. While it is evident that these etiologies predominantly lead to focal seizures, secondary generalization is also possible.

Table 7.1: Patient demographics and clinical characteristics

	Generalized epilepsy		
Characteristic	**Primary**	**Secondary**	**Overall**
n (%)	20 (54%)	17 (46%)	37 (100%)
Mean age, years (SD)*	36 (14)	45 (18)	40 (17)
Sex			
Male	12 (60%)	10 (59%)	22 (60%)
Female	8 (40%)	7(41%)	15 (40%)
Self-reported ethnicity			
African	18 (90%)	16(94%)	34 (91%)
Indian	1 (5%)	0 (0%)	1 (3%)
Caucasian	0 (0%)	1 (6%)	1 (3%)
Not specified	1 (5%)	0 (0%)	1 (3%)
Chronic medication			
Amitriptyline	2 (10%)	0 (0%)	2 (5%)
Risperidone	0 (0%)	2 (12%)	2 (5%)
Rivaroxaban	0 (0%)	1 (6%)	1 (3%)
ARVs	1 (5%)	0 (0%)	1 (3%)
Neomercazole	0 (0%)	1 (6%)	1 (3%)
Secondary stroke prevention and cardiac treatment*	0 (0%)	1 (6%)	1(3%)
Metformin and Amlodipine	0 (0%)	1 (6%)	1 (3%)
None	17 (85%)	11 (65%)	28 (75%)
Mean Seizures per month (SD)	0.9 (1.3)	0.2 (0.4)	0.6 (1.1)

*SD is standard deviation.
*Stroke prevention and cardiac treatment includes aspirin, simvastatin, carvedilol and spironolactone.

Laboratory results are shown in Table 7.2. Patients had been on sodium valproate therapy for over two years, with dosages exceeding one gram per day. The sodium valproate levels remained within the therapeutic range, and the mean ammonia levels were consistently within normal limits across all groups examined. The therapeutic range, as specified by the NHLS, was included at the bottom of the table.

Table 7.2: Laboratory results of entire study sample (n=37)

	Generalized Epilepsy		
Characteristic	**Primary**	**Secondary**	**Overall**
n (%)	20 (54%)	17 (46%)	37 (100%)
Time on sodium valproate, years (SD)	2.2 (2.7)	4.2 (4.6)	3.1 (3.9)
Sodium valproate dosage per day,mg (SD)	1140 (297)	1070 (193)	1108 (257)
Sodium valproate levels, umol/L (SD)	478 (210)	421 (186)	452 (201)
Ammonia levels, umol/L (SD)	34 (14)	34 (32)	34 (24)

Numerical values presented as mean and (SD) is standard deviation
*NHLS reference range for VPA levels is 346-693 umol/L
*NHLS reference range for ammonia levels are 11-35umol/L

The proportion of patients with raised ammonia levels are illustrated in Figure 7.2. Notably, patients with PGE exhibited a higher proportion of raised ammonia levels. However, the significance of this finding was not corroborated by existing literature.

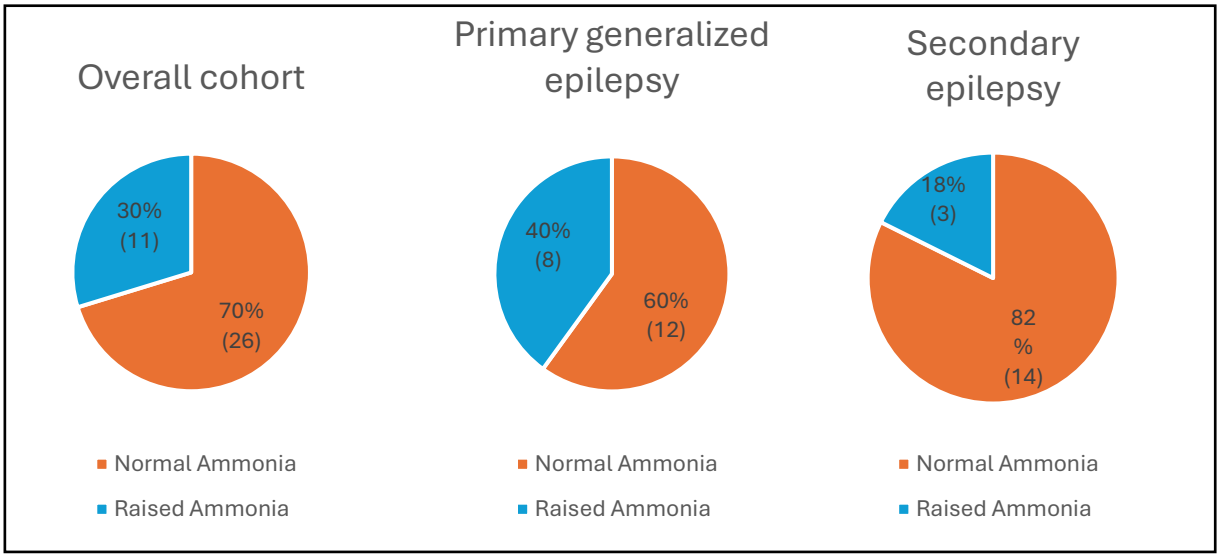
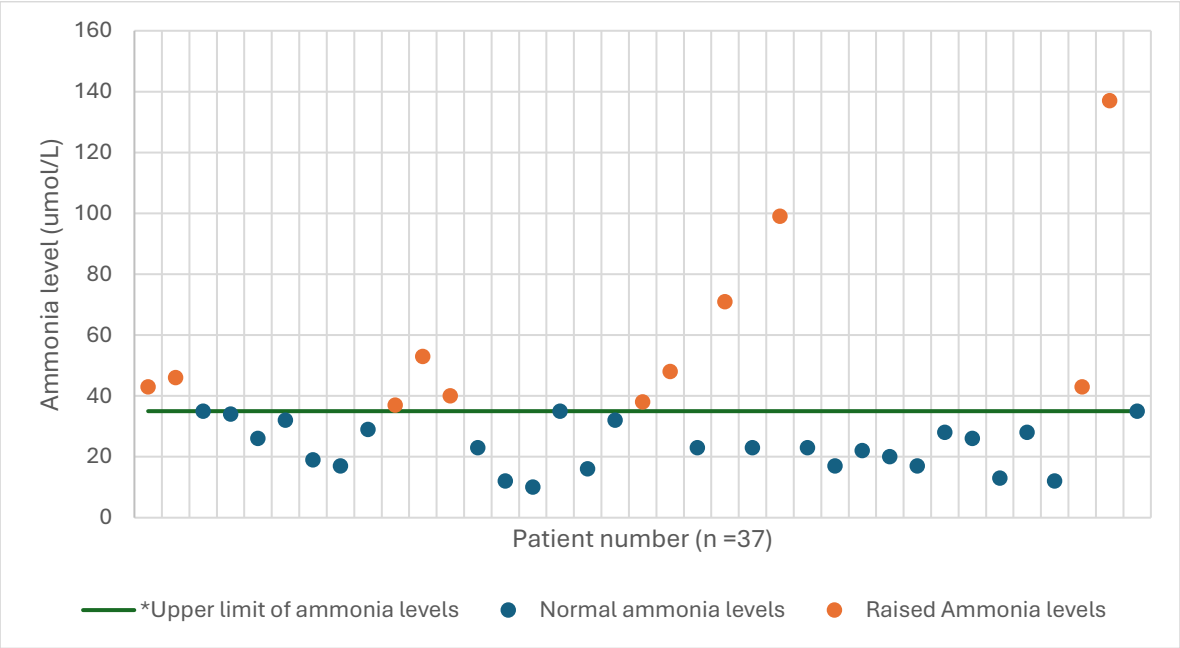


Figure 7.2: Proportion of the entire study with raised ammonia levels

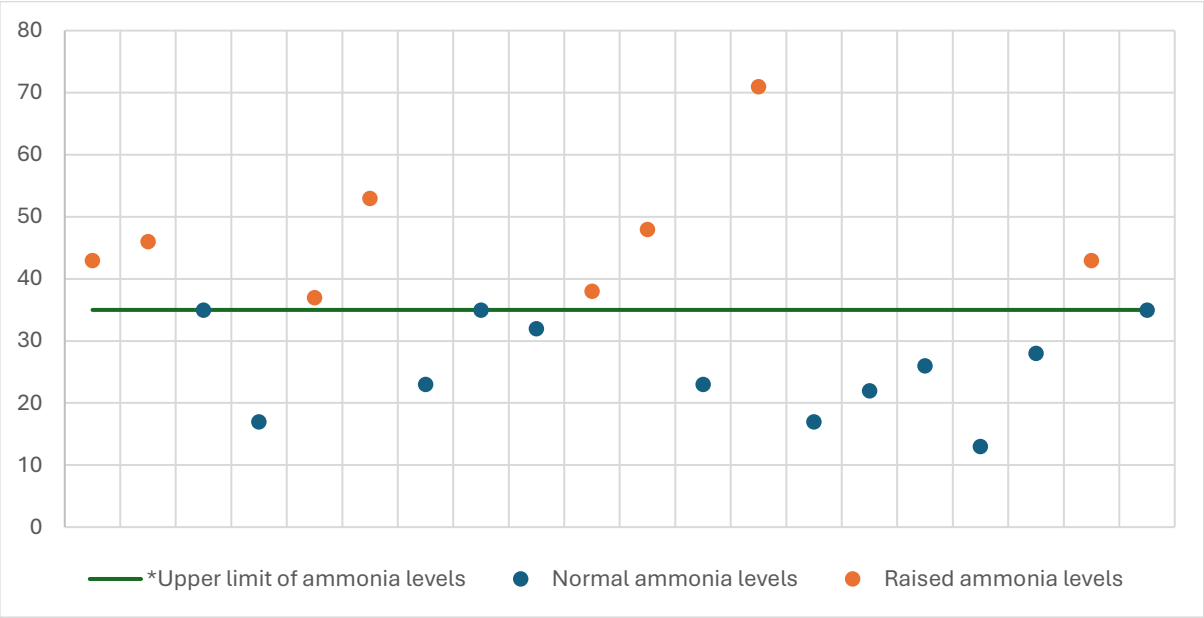
The scatter chart in Figure 7.3 illustrates the serum ammonia levels of the entire study (n=37). 11 patients had elevated serum ammonia levels.



*According to the NHLS the upper limit is 35 umol/L

Figure 7.3: Ammonia levels per patient

A comparative scatter chart in Figure 7.4 demonstrated serum ammonia levels in patients with PGE. This figure illustrates that 8 out of the 20 patients exhibited elevated serum ammonia levels. Additionally, the average ammonia level among patients with primary epilepsy was 34.3 umol/L (±13.7). The highest recorded level was 71umol/L.



* According to the NHLS the upper limit is 35 umol/L

Figure 7.4: Primary epilepsy patients and their ammonia levels

7.2. Overall study versus raised ammonia levels

Among the 37 patients in the research study, eleven had elevated ammonia levels. This group was younger and predominantly male compared to the overall study. Both groups experienced poor seizure control. Patients with elevated ammonia levels had been on sodium valproate for a shorter duration but at a higher dosage. (Table 7.3)

Two of the eleven patients with raised ammonia levels were on other medications. One was taking amitriptyline and the other risperidone. Patients with elevated ammonia levels were not on antiretroviral therapy or tuberculosis treatment. Those who used these treatments had abnormal liver function tests, or their ammonia samples were rejected due to technical errors. Consequently, these patients were excluded from the final group with raised serum ammonia levels.

Table 7.3: Comparison of the entire study with patients with raised ammonia (n=11)

Characteristic	Raised ammonia	Overall
n (%)	11 (30%)	37 (100%)
Mean age, years (SD)	35 (15)	40 (17)
Sex		
Male	9 (82%)	22 (60%)
Female	2 (18%)	15 (40%)
Self-reported ethnicity		
African	10 (91%)	34 (91%)
Indian	1 (9%)	1 (3%)
Caucasian	0 (0%)	1 (3%)
Not specified	0 (0%)	1 (3%)
Chronic medication		
Amitriptyline	1 (9%)	2 (5%)
Risperidone	1 (9%)	2 (5%)
Rivaroxaban	0 (0%)	1 (3%)
ARVs	0 (0%)	1 (3%)
Neomercazole	0 (0%)	1 (3%)
*Secondary stroke prevention and cardiac treatment	0 (0%)	1 (3%)
Metformin and Amlodipine	0 (0%)	1 (3%)
None	9 (82%)	28 (75%)
Seizure control, n (%)		
Seizure free	4 (36%)	13 (35%)
Not seizure free	7 (64%)	24 (65%)
Mean Seizures per month (SD)	0.5 (0.5)	0.6 (1.1)
Mean time on sodium valproate, years (SD)	2.6 (2.4)	3.1 (3.9)
Mean sodium valproate dosage per day, mg (SD)	1127 (242)	1108 (257)
***Mean sodium valproate levels**, umol/L (SD)	390 (192)	452 (201)
***Mean ammonia Levels**, umol/L (SD)	58 (31)	34 (24)

*Numerical values presented as mean and (SD) standard deviation
*Stroke prevention and cardiac treatment includes aspirin, simvastatin, carvedilol and spironolactone
*NHLS reference range for VPA levels is 346-693 umol/L
*NHLS reference range for ammonia levels are 11-35umol/L

Data was collected during the clinic visit. Any recent symptoms of nausea and/or vomiting, new neurological deficits, and a recent increase in seizure frequency were documented. Families and caregivers were asked if they observed any recent changes in the patient's mental state. Among the group of eleven patients with hyperammonemia, only one patient reported a recent increase in seizure frequency while on sodium valproate. Additionally, a change in cognition was documented in another patient. The remaining nine patients did not report any alterations in their clinical presentation.

Table 7.4: Change in clinical presentation of patients with raised ammonia

Change in Clinical Presentation	**Number of Patients (n=11)**
Seizure frequency increase	1
Change in cognition	1
Nausea/vomiting	0
Change in neurological examination	0

Family members or caregivers accompanying patients were inquired about the monthly seizure frequency. If the frequency of seizures remained stable or showed a decrease, it was not recorded as an increase in seizure activity. This documentation was intended to assess whether patients experienced uncontrolled seizures, possibly due to elevated ammonia levels while being treated with sodium valproate.

The average number of seizures per month in patients with raised ammonia was illustrated in figure 7.5. The majority had 0-0.24 seizures per month.

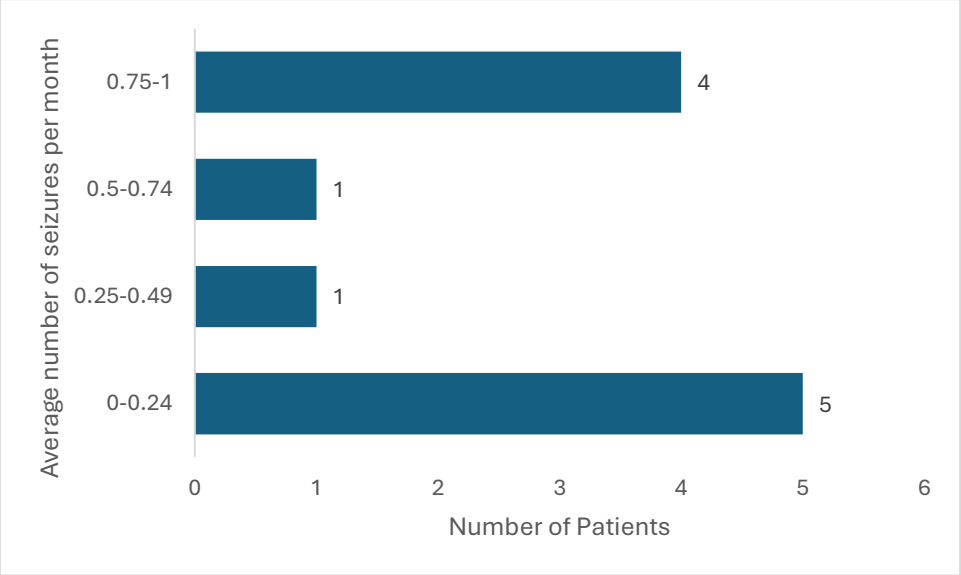


Figure 7.5: Number of Seizures per month for patients with raised ammonia levels

8. DISCUSSION

The occurrence of sodium valproate-induced hyperammonemia is predominantly documented in international journal articles. No South African studies were identified for comparison; thus, this research primarily references three studies conducted by Gomcelli *et al*. (2007), Cheng *et al*. (2012), and Tseng *et al*. (2014). Table 11.1 in Appendix 4 presents all data discussed herein (12,21,22).

This study mainly focused on the ammonia levels of epileptic patients on monotherapy with VPA; however, a few other characteristics and associations have also been observed. These are only observations and may need additional studies to be confirmed.

Demographics

Compared to the three studies mentioned above, whose mean ages were 41, 36 and 38 years, the mean age in this study was 35 years for patients with raised ammonia. Like Cheng *et al*. and Tseng *et al*. who had a higher percentage of men (75% and 66%) compared to women, this study also has predominantly male patients (82%). This contrasts Gomcelli *et al*., who had fewer men (43%) than women. Gomcelli *et al*. had 57% of patients with primary

generalised epilepsy. Our research study demonstrated similar data with 64% of patients having primary generalised epilepsy. (Page 4, appendix 4) Tseng *et al*. predominantly had secondary epilepsies at 61% and Cheng *et al*. did not specify the type of epilepsy (12,21,22).

Duration of VPA therapy

This study sample has been on VPA therapy for a mean duration of 2.6 years. The mean duration on VPA for the various studies are significantly different, Gomcelli *et al*. and Cheng *et al*. had a mean duration of less than one year at 0.7 (36 weeks) and 0.1 years (five weeks), while Tseng *et al*. had a mean duration of eight years (12,21,22). Tseng *et al*. and Cheng *et al*. showed no correlation between duration on VPA and raised ammonia levels. Cheng *et al*. noticed that patients on VPA developed hyperammonemia from two to sixty days from the initiation of the drug (12,21,22).

VPA dosage

The mean VPA dosage in patients with hyperammonemia was slightly higher at 1127mg. The mean VPA dosage was comparable in all the studies that were conducted. Gomcelli and Tseng *et al*. documented mean VPA dosages as 1000 mg per day, Cheng *et al*. calculated 980mg. Tseng *et al*. documented that for every milligram VPA was increased, the ammonia levels went up by 0.01%. There was a linear relationship between VPA dose and serum ammonia levels. However, Cheng *et al*. and Gomceli *et al*. reported no correlation between the dose of VPA and ammonia levels (12,21,22).

Serum VPA levels

The mean sodium valproate levels were therapeutic for their laboratory reference range, in all four studies (12,21,22).

Serum ammonia levels

The mean serum ammonia levels were elevated in all three studies that was used as comparison. The first two articles used encephalopathic patients but the last study, included ammonia levels in patient that were inter-ictal (12,21,22). This differed from the current study as patients were primarily selected if they were on VPA monotherapy rather than their cognitive state. Serum ammonia levels were raised in 11 of the 37 patients selected. The larger study done by Tseng *et al*. (n=158), showed that 27% on VPA monotherapy had raised serum ammonia levels (level

>54.6umol/L), and 5.1% of those patients had severe hyperammonemia (level >88.1 umol/L). The mean ammonia level in those with raised ammonia was shown to be 58umol/L (12).

In patients diagnosed with encephalopathy, there was a significant clinical correlation with elevated ammonia levels (21,22). However, a study conducted on inter-ictal patients, who were tested between seizure episodes, revealed that these individuals remained asymptomatic despite the presence of raised ammonia levels (12).

Seizure control

In this research study, 36% was seizure free and 64% had seizures. In the study by Gomcelli *et al*., none of the patients were seizure-free, while Tseng *et al*. reported that 65% of their cohort continued to experience seizures but the seizure frequency was not aggravated in those with raised ammonia levels. Cheng *et al*. did not comment on seizure control. The reason for not commenting on seizure control, can be explained by their patient selection, including those with psychiatric illnesses and use of VPA as prophylactic treatment (12,21,22).

Seizure type

Tseng *et al*. had the largest sample size but predominantly included secondary epilepsy at 61% whereas Gomcelli *et al*. and this research study had more patients with PGE in the sample description. Gomcelli *et al*. had 4 patients (57.14%) whereas this study included 7 (64%) patients with primary epilepsies (12,21,22).

Liver functions

All the studies mentioned above had normal LFTs (12,21,22). The current study only included patients with normal LFT's.

Other medications

Other medications used in patients with raised ammonia was amitriptyline and risperidone. Tseng *et al*. reported that anti-psychotic medication can be related to raised ammonia in 70% of patients (n=7). VPA has been reported to have drug interactions with risperidone (12).

Clinical presentation

The three studies noted that patients had behavioural changes, decreased concentration, nausea, ataxia and increased seizure frequency. Their patients were reported to be more

symptomatic as they mostly tested patients that were encephalopathic (12,21,22). In this study, very few patients reported a change in cognitive state or change in seizure frequency. Tseng *et al*.'s patients with hyperammonemia were mostly asymptomatic (12).

This can be explained by the fact that these patients did not present with any new acute symptoms to suggest a change in their condition, but they were selected whilst attending their scheduled follow up appointment at the epilepsy outpatient department.

Management of VIH

According to Tseng *et al*., patients with symptomatic hyperammonemia had a benign course and rapidly recovered after correction of blood ammonia with treatment such as lactulose therapy. In patients with severe and symptomatic hyperammonemia, it was necessary to decrease the VPA dosage or to discontinue the drug completely (12). Cheng *et al*. reported that time to recovery from VHE ranged from 1 to 14 days (22).

9. LIMITATIONS

Several limitations were identified in this research study. Firstly, the sample size was small and may not accurately represent ammonia levels in state hospitals. Additionally, the process of obtaining serum ammonia levels was labor-intensive and susceptible to human error and procedural breakdowns. There was also a potential selection bias, as only patients from tertiary hospitals were included, whereas eligible patients from local clinics and primary and secondary hospitals were excluded.

Many epileptic patients were unaccompanied by family members, which may have resulted in inaccurate data collection regarding changes in their cognition. Additionally, a standardized test was not utilized to assess cognitive changes before and after the initiation of VPA. Electroencephalograms (EEGs) can aid in diagnosing patients with VIH and ensuring that those with secondary epilepsy have generalized epilepsy. This is a limitation when describing patients with encephalopathic features, as EEG findings were not included in the study.

10. CONCLUSION

This study determined that 30% of patients undergoing sodium valproate monotherapy in Johannesburg state hospitals exhibited elevated ammonia levels. Two patients presented with altered neurological symptoms, suggesting the potential need for discontinuation of the medication.

11. APPENDICES

11.1. Appendix 1: Mechanism of action of sodium valproate

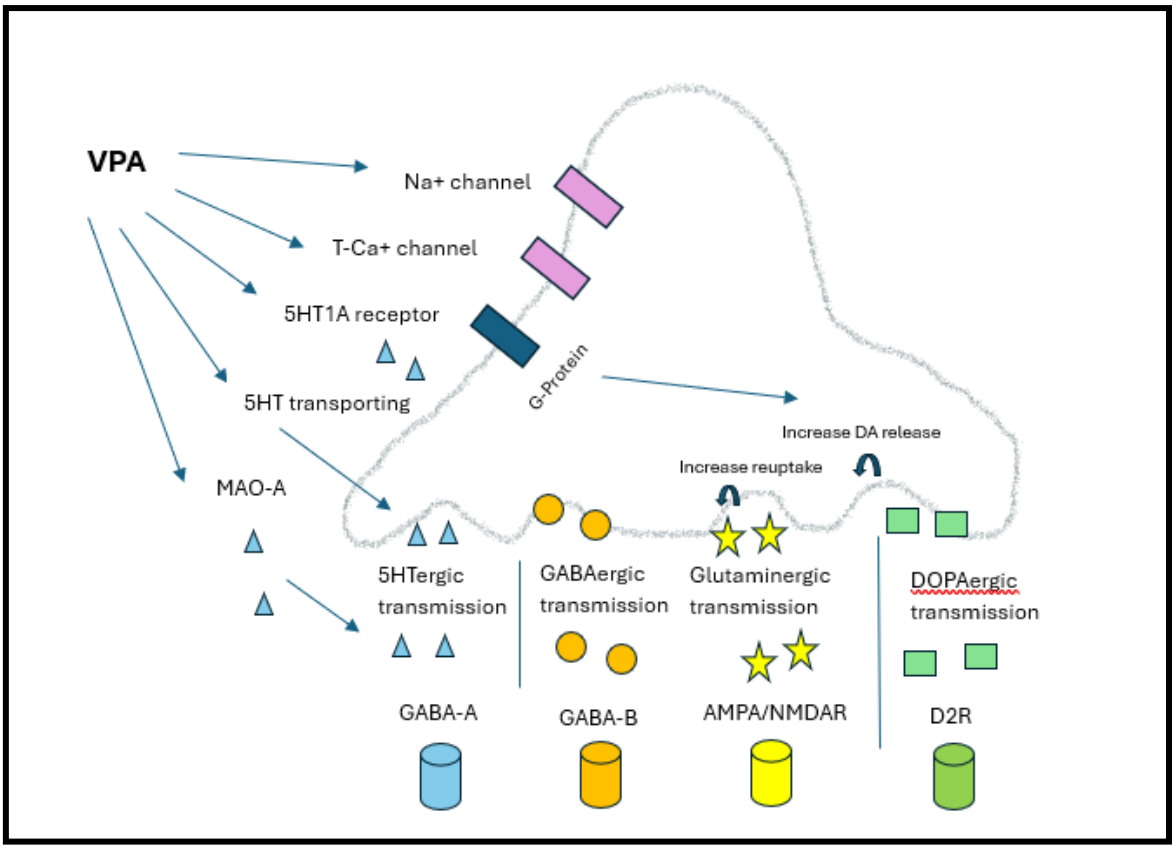


Figure 11.1: Mechanism of action of sodium valproate adapted from Romoli *et al.* (27)

Valproate effects on synaptic cleft and intracellular pathways. The increase in GABAergic transmission pairs with a modulation other aminergic systems; the excitatory ionic channels, such as calcium and sodium channels, are negatively modulated, while potassium currents are enhanced. Moreover, epigenetic mechanisms influence receptor expression and neuroprotective pathways. Legend. AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid –AMPA- receptor; BDNF: brain derived neurotrophic factor; DA: dopamine; D2R: dopamine D2 receptor; GABA: gaba-amino-butirric acid; GABAR: GABA receptors; GABA-A: GABA receptor type A; GABA-B: GABA receptor type B; HSPs: heat shock proteins; HSP-70: heat shock protein 70; K^+: potassium; IDO: indoleamine 2,3 dioxygenase; MAO-A: mono-amines oxidase type A; M-channel: low-threshold noninactivating voltage-gated potassium current; MMP-9: metalloproteinases-9; Na^+ channel: sodium channel; NMDAR: N-metyl-D-aspartate -NMDA- receptor; NR2A: 2A subunit of NMDAR; NR2B: 2B subunit of NMDAR; Par-4: prostate apoptosis response-4; T-Ca^+ channel: low-threshold T-calcium channel; TNF-α: tumor necrosis factor α; VPA: valproate; 5HT: serotonin.

11.2. Appendix 2: Effects of other medication on sodium valproate

Table 11.1: Effects of other medication on sodium valproate(27)

Drugs decreasing VPA concentration	**Drugs increasing VPA concentration**
Carbamazepine:Induces hepatic metabolism	Aspirin (high dose): protein binding displacement/ decreased intrinsic clearance
Carbapenem antibiotics: Multiple mechanisms	Cimetidine: inhibits hepatic metabolism
Lamotrogine: Induces hepatic metabolism	Macrolides: Unknown mechanism
Phenytoin: Induces hepatic metabolism	Felbamate: inhibits hepatic metabolism
Phenobarb: Induces hepatic metabolism	Risperidone: unknown mechanism
Rifampicin: Induces hepatic metabolism	
Ritonavir: Induces hepatic metabolism	
Sevelamer: decreases absorption of VPA	

11.3. Appendix 3: Mechanism of VIH

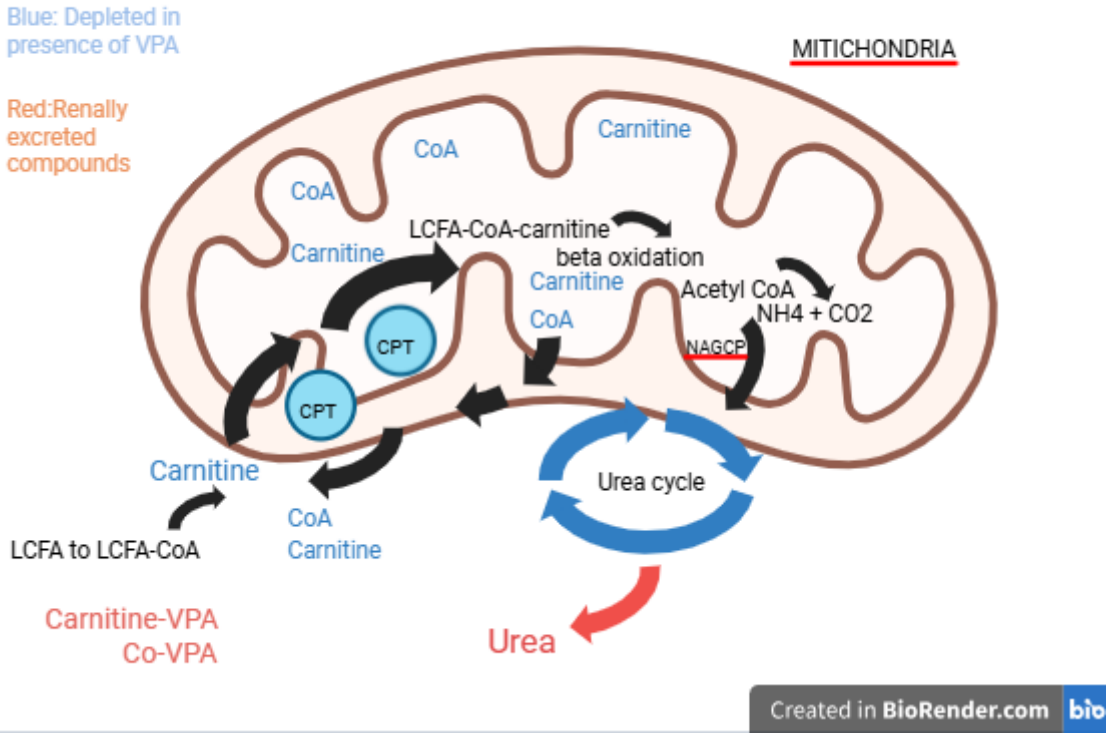


Figure 11.2: Mechanism of raised ammonia in VPA treatment adapted from Biorender (28)

In hepatic mitochondria, valproic acid is believed to cause an accumulation of ammonia by reducing free carnitine and co-enzyme A, which bond to valproic acid and are then either renally excreted or sequestered in the mitochondrial matrix. Loss of carnitine prevents importation of long-chain fatty acids into the mitochondrial matrix for metabolism. Loss of co-enzyme A prevents the beta-oxidation of fatty acids into acetyl-co-enzyme A, which is a substrate of *N* -acetyl-glutamate, the required activator of the initial enzyme in the urea cycle. CoA:Co-enzyme A, CP:Carbamoyl phosphate synthetase 1, CPT: Carnitine plamitoyltransferase, LCFA: Long chain fatty accid, NH4: Ammonia, NAG:N-Acetyl-glutamate.

11.4. Appendix 4: Patients with raised ammonia compared to three other studies.

Table11.2: Comparison of raised ammonia cohort to other studies

	Patients with raised ammonia (n= 11)	Gomcelli *et al*., 2007 (n=7)	Cheng *et al*., 2012 (n=20)	Tseng *et al*., 2014 (n=158)
Mean age, years (SD)	35 (15)	41	36	38
Sex; n (%)				
Male	9 (82%)	3 (43%)	15(75%)	104(66%)
Female	2 (18%)	4 (57%)	5 (25%)	54(34%)
Total	11	7	20	158
Seizure control, n (%)				
Seizure free	4 (36%)	0 (0%)	Not specified	54 (35%)
Not seizure free	7 (64%)	7 (100%)		103 (65%)
Type of epilepsy, n (%)				
Primary	7 (64%)	4 (57%)	Not specified	62 (39%)
Secondary	4 (36%)	3 (43%)		96 (61%)
ASM administered, n (%)				
Monotherapy with VPA (SD)	11 (100%)	4 (57%)	14 (70%)	31 (20%)
Combination Therapy (SD)	0 (0%)	3 (43%)	6 (30%)	127 (80%)
Dose of VPA mg/day (range)	1127 (800-1600)	1000 (500-1500)	980 (762-1198)	1000 (787-1500)
Mean VPA level, umol/L (SD) *Standard deviation was not available in the studies	390 (192)	481*	541*	406*
Duration on VPA, in years (SD)	2.6 (2.4)	0.7	0.1	8 .0
Mean serum ammonia level, umol/L(SD)	58 (31)	75	138	44
Patients included	Epileptics	Epileptics	Epileptics and MHCU* and prophylactic ASM	Epileptics
LFTs	Normal	Normal	Normal	Normal

*Numerical values are presented as mean and SD (standard deviation)
*MHCU (Mental health care user)
*NHLS reference range for VPA levels is 346-693 umol/L
*NHLS reference range for ammonia levels are 11-35umol/L

11.5. Appendix 5: Data collection sheet

Data collection sheet: Date:

Patient number	
Age	
Sex	
Self-reported ethnicity	

- **Epilepsy Classification (Primary/Secondary):**
- **Duration on Sodium Valproate (years)**:
- **Dose of Sodium Valproate (mg per day-**
- **Other chronic medication/s**:
- **Clinical presentation**:

Change in cognition ☐
Nausea/vomiting ☐
Neurological fallout ☐
Change in seizure frequency ☐

Investigations:

	Value	**Reference range** (Used by NHLS)
Sodium valproate level (umol/L)		346-693 umol/L, toxic>1040
Liver function tests		ALT (7-35umol/L) AST (13-35umol/L) GGT (<40umol/L)
Ammonia levels		11-35 umol/L
Seizures per month		-

11.6. Appendix 6: patient information sheet

Patient information sheet

What will happen if I agree to take part in the study?

After explaining the study to you, I will be asking you about your history of epilepsy. It will include when you were diagnosed and for how long you are taking Epilim. I will also be taking 1 purple and 1 yellow tube of blood and ask you to elaborate on the frequency of your fits e.g. how many fits do you usually have per month. All the results will be available at your next clinic follow up visit.

What are the side effects or risks of taking part in the study?

The only procedure that will be done is that blood that will be drawn and might be uncomfortable.

What are the benefits of taking part in the study?

The study will help determine if we need to do ammonia levels in all patients on Epilim. If the study shows a link between number of fits and the blood levels, then it can be recommended that doctors do such tests in the clinic. The overall goal will be to improve quality of life by having controlled epilepsy.

Is there reimbursement for taking part in the study?

You will not be paid to participate in the study and there will not be any costs to you for the investigations that are done.

Do I have a choice when taking part in the study?

This study is voluntary, and you will not be forced to partake in it. If you agree to the study, you can take the information sheet with you, and I will ask you to sign an informed consent form. If you decide to discontinue participation in this study, you may do so freely at any time without providing a reason. The discontinuation of participation will not affect the care and treatment that you receive from the clinic or doctor in any way.

Will the information be kept confidential if I take part in the study?

All information will be kept confidential, and participation will be anonymous. All the clinical information and results of special investigations will be kept in the respective hospitals and outpatient departments. Only authorized personnel will have access to the files. The study information will only include a participant number and will not include your name.

Data may be published in scientific journals but will not include any information whereby you can be identified as a participant. The information and data will be kept for two years if published and six years if not published, after which all information will be destroyed. If the researchers want to share the data with other researchers for academics or further research purposes, written permission will be obtained from the Human Research ethics committee (HREC).

Who is organizing and funding the research project?

Dr. Kareli Jonker will be organizing the study in collaboration with my supervisor, prof.G.Modi. The department of neurology will be responsible for the funding of the investigations and will not be used for commercial gain.

Who can I call if I have any questions?

If you have any questions, concerns or if you need more information with regards to the study, you can call Dr. K.Jonker at 072 316 4447 or e-mail at kareli@live.co.za

Or

Human Research Ethics committee (medical), university of Witwatersrand -Prof P Cleaton Jones, tel: (011) 717 2301, email: peter.cleaton-jones@wits.ac.za.
-Ms.Ndlovu, Mr Mkansi, Mr. Moeng at 011 717 2700/2656/1234/1252, e-mail: zanele.ndlovu@wits.ac.za, rhulani.mkansi@wits.ac.za, lebo.moeng@wits.ac.za.

11.7. Appendix 7: Informed consent form

Informed consent form

Title of study: Ammonia levels in patients with generalized Epilepsy that are on sodium valproate monotherapy.

I confirm that I have been informed by Dr.K.Jonker/ other doctor about the nature of the study.

I have read the information sheet and understood the information presented to me. I also had the opportunity to ask questions.

I understand that my participation is voluntary and that I am allowed to withdraw from the study at any time without giving a reason and without my medical care or rights being affected.

I understand that Dr.K. Jonker/ other doctor will ask me questions about my history with epilepsy and draw blood for certain blood levels.

I agree to take part in the above-mentioned study. I hereby give consent to the consultation and to have my blood taken. I consent to have the results used in a research study.

Name and surname of participant:……

Signature:……

Thumb print if not able to write:

Date:…….

Next of kin: Relation……

Name and surname…….

Signature…….

Date….....

Translator explaining informed consent (if applicable)

Name and surname…….

Signature…….

Date….....

Witness 1:

Name and surname…….

Signature…….

Date….....

Witness 2:

Name and surname…….

Signature…….

Date….....

11.8. Appendix 8: Ethics clearance form

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Dr K Jonker

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220701

NAME: (Principal Investigator)	Dr K Jonker
DEPARTMENT:	School of Clinical Medicine Department of Neurosciences Division of Neurology Medical School University
PROJECT TITLE:	*Ammonia level in patients with primary generalized epilepsy that are on sodium valproate monotherapy*
DATE CONSIDERED:	2022/07/29
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr KD Jivan
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/03/29

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **July** and therefore reports and re-certification will be due in the month of **July** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator — Date

Jonker — 2023/03/31

11.9. Appendix 9: Plagiarism form

Turn-it-in report

KJonker_MMED_FinalV5-2.docx

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12. REFERENCES

1. Nolan SJ, Sudell M, Weston J, Tudur Smith C, Marson AG. Antiepileptic drug monotherapy for epilepsy: A network meta-analysis. Cochrane Database of Systematic Reviews. 2014 Dec 2;2014(12).
2. Falco-Walter J. Epilepsy-Definition, Classification, Pathophysiology, and Epidemiology. Semin Neurol. 2020 Dec;40(6):617-623.
3. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, *et al.* ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017 Apr;58(4):512-521.
4. Owolabi LF, Sale S, Owolabi SD, Nalado A, Umar M, Taura AA. Electroencephalography abnormalities in generalized epilepsy and their predictors: A multicenter experience. Ann Afr Med. 2018 Apr-Jun;17(2):64-69.
5. Gonzalez-Viana E, Sen A, Bonnon A, Cross JH; Guideline Committee. Epilepsies in children, young people, and adults: summary of updated NICE guidance. BMJ. 2022 Jul 5;378:o1446.
6. Keuler N, Johnson Y, Coetzee R. A description of sodium valproate, lamotrigine and levetiracetam consumption in the Western Cape public sector. S Afr Fam Pract (2004). 2022 Jun 2;64(1):e1-e7.
7. Perucca E. Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. CNS Drugs. 2002;16(10):695-714.
8. Patsalos PN, Berry DJ, Bourgeois BF, Cloyd JC, Glauser TA, Johannessen SI, *et al*. Antiepileptic drugs--best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia. 2008 Jul;49(7):1239-76.
9. Chanvanichtrakool M, Schreiber JM, Chen WL, Barber J, Zhang A, Ah Mew N, *et al*. Urea Cycle Disease Consortium. Unraveling the Link: Seizure Characteristics and Ammonia Levels in Urea Cycle Disorder During Hyperammonemic Crises. Pediatr Neurol. 2024 Oct;159:48-55.
10. Rangroo Thrane V, Thrane AS, Wang F, Cotrina ML, Smith NA, Chen M, *et al*. Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. Nat Med. 2013 Dec;19(12):1643-8.
11. Nanau RM, Neuman MG. Adverse drug reactions induced by valproic acid. Clin Biochem. 2013 Oct;46(15):1323-38.

12. Tseng YL, Huang CR, Lin CH, Lu YT, Lu CH, Chen NC, *et al*. Risk factors of hyperammonemia in patients with epilepsy under valproic acid therapy. Medicine (Baltimore). 2014 Sep;93(11):e66.
13. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012
14. Baddour E, Tewksbury A, Stauner N. Valproic acid-induced hyperammonemia: Incidence, clinical significance, and treatment management. Ment Health Clin. 2018 Mar 26;8(2):73-77.
15. Duman B, Can KC, Ağtaş-Ertan E, Erdoğan S, İlhan RS, Doğan Ö, *et al*. Risk factors for valproic acid induced hyperammonemia and its association with cognitive functions. Gen Hosp Psychiatry. 2019 Jul-Aug; 59:67-72.
16. Häberle J. Clinical and biochemical aspects of primary and secondary hyperammonemic disorders. Arch Biochem Biophys. 2013 Aug 15;536(2):101-8.
17. Badawy AA, Elghaba R, Soliman M, Hussein AM, AlSadrah SA, Awadalla A, *et al*. Chronic Valproic Acid Administration Increases Plasma, Liver, and Brain Ammonia Concentration and Suppresses Glutamine Synthetase Activity. Brain Sci. 2020 Oct 21;10(10):759.
18. Agrawal. Valproate-Induced Non-Hepatic Hyperammonaemic Encephalopathy: A Rare Complication of Chronic Valproate Therapy. International Journal of Clinical Pediatrics. 2012.
19. Chicharro AV, de Marinis AJ, Kanner AM. The measurement of ammonia blood levels in patients taking valproic acid: looking for problems where they do not exist? Epilepsy Behav. 2007 Nov;11(3):361-6.
20. Campos MSA, Ayres LR, Morelo MRS, Carizio FAM, Pereira LRL. Comparative efficacy of antiepileptic drugs for patients with generalized epileptic seizures: systematic review and network meta-analyses. Int J Clin Pharm. 2018 Jun;40(3):589-598.
21. Gomceli YB, Kutlu G, Cavdar L, Sanivar F, Inan LE. Different clinical manifestations of hyperammonemic encephalopathy. Epilepsy Behav. 2007 Jun;10(4):583-7.
22. Cheng M, Tang X, Wen S, Yue J, Wang H. Valproate (VPA)-associated hyperammonemic encephalopathy independent of elevated serum VPA levels: 21 cases in China from May 2000 to May 2012. Compr Psychiatry. 2013 Jul;54(5):562-7.
23. Britton JW, Frey LC, Hopp JL, Korb P, Koubeissi MZ, Lievens WE, *et al*. Electroencephalography (EEG): An Introductory Text and Atlas of Normal and Abnormal Findings in Adults, Children, and Infants [Internet]. St. Louis EK, Frey LC, editors. Chicago: American Epilepsy Society; 2016.

24. Guerrini R, Marini C, Barba C. Generalized epilepsies. Handb Clin Neurol. 2019;161:3-15.
25. Kifune A, Kubota F, Shibata N, Akata T, Kikuchi S. Valproic acid-induced hyperammonemic encephalopathy with triphasic waves. Epilepsia. 2000 Jul;41(7):909-12.
26. Butterworth RF, Giguère JF, Michaud J, Lavoie J, Layrargues GP. Ammonia: key factor in the pathogenesis of hepatic encephalopathy. Neurochem Pathol. 1987 Feb-Apr;6(1-2):1-12.
27. Tesoro E, Brophy GM, Cohen H. Basicmedical Key. 2017;19.
28. Carr RB, Shrewsbury K. Hyperammonemia due to valproic acid in the psychiatric setting. Am J Psychiatry. 2007 Jul;164(7):1020-7.