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Assessment of Plasma Aminotransferases, Alkaline Phosphatase and Albumin in Epileptic Children Treated with Sodium Valproate

تقييم نشاط انزيمات الامينوترانسفيريز، الكالين فسفاتيز والالبومين في بلازما الدم لدى الاطفال المصابين بالصرع المعالجون بفالبرات الصوديوم

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الآيــة

{اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ * خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ * اقْرَأْ وَرَبُّكَ الْأَكْرَمُ * الَّذِي عَلَّمَ بِالْقَلَمِ * عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ}

صدق الله العظيم

سورة العلق(1–5)

Dedication

To soul of my father

To my mother

To my friends

I dedicate this works

Acknowledgments

Graduate and thanks fullness to Allah for giving me strength and ability to complete this work. I would like to express my sincere thanks and gratitude to my supervisor Dr.Ghada Elfadil for supervising and support me for completing this work. Also I would like to thanks the staff of Mohammed Elamin Hamid hospital to help me in collection of the samples moral support. Grateful thanks to all children parents who participated in this work. Finally to everyone helped me especially my family and friends.

Abstract

Background and Aim of the study: anti-epileptic drug therapy such as sodium valproate (VPA) may had adverse effect on hepatic function, liver enzymes reflect liver dysfunction and may be useful in detecting early hepatocellular injury. The aim of this study was to assess of plasma Aminotransferases (AST, ALT), Alkaline Phosphatase (ALP) activities, Total protein (TP) and Albumin (Alb) concentrations in epileptic children used sodium valproate.

Materials and M**ethods**: this was a comparative cross-sectional study enrolled of 80 Sudanese children; 40 epileptic children (22 boys, 18girl), age range from (4-17) years from Mohammed Elamin Hamid hospital in Khartoum state, 40 children apparently health as control group. The plasma was separated and used to measure total protein, albumin concentrations and AST, ALT and ALP activities, they were assayed biochemically using Mindray full automation, data was analyzed by SPSS version 20.

Results: showed there was significant increase in means of plasma AST (30.4± 12.1 U/L), ALT (17± 4.2 U/L) and ALP activities (232±103.9 U/L,) $P \leq 0.05$ respectively in epileptic children used VPA when compared with means in control group AST (25.1±5.7 U/L), ALT (17 ± 4.2 U/L), ALP (189.3 ± 20.4 U/L). Insignificant change in means of plasma total protein (7.3± 0.5 g/dL) and albumin concentrations (4±0.3 g/dL) in patient when compared with control (Alb (40.8 ± 0.3 g/dL), TP (74.6 ± 0.5 g/dL).

Conclusion: plasma Aminotransferases (ALT, AST) and alkaline Phosphatase activities increase among Sudanese epileptic children on VPA treatment in this study.

المستخلص

الخلفية والهدف من البحث الأدوية المضادة للصرع مثل فالبرات الصوديوم الزي له تأثير سالب على وظائف الكبد وأنزيمات الكبد قد تعكس اختلال وظيفة الكبد وقد تكون مفيدة في الكشف عن الإصابة المبكرة لخلايا الكبد . .الهدف هو تقييم نشاط إنزيمات الامينوترانسفيريز ،الكالين فسفاتيز،البروتين الكلي والألبومين في بلازما الدم في الأطفال المصابين بالصرع الذين يستخدمون فالبرات الصوديوم في العلاج

المواد والطرق المستخدمة : أجريت هذه الدراسة في ولاية الخرطوم (مستشفى محمد الأمين حامد للأطفال) في الفترة من أبريل حتي يوليو وقد تم اختيار 80 مشارك منهم 40 مشارك من الجنسين (ذكور/ إناث) مصابين بالصرع و40 منهم ظاهريا أصحاء (كنترول).تم الحصول علي بلازما الدم بواسطة جهاز الطرد المركزي لفحص البروتين الكلي، الزلال ،النين ترانسفيريز، سبارتيت ترانسفيريز ،الكالين فسفاتيز وتم الفحص بواسطة جهاز اوتوماتيك (مندري) وتم تحليل البيانات بواسطة الحزمة الإحصائية الاجتماعية الإصدارة 20

النتائج : كانت النتائج ان هناك زيادة معنوية في متوسطات سبارتيت ترانسفيريز(30.4 ± 12.1 وحدة/لتر)، النين ترانسفيريز(17 ± 4.2 وحدة /لتر) ، الكالين فسفاتيز(232± 103.9 وحدة/لتر) القيمة الاحتمالية اقل من او تساوي 0.05 علي التوالي عند الأطفال المصابين بالصرع الزين يستخدمون فالبرايت الصوديوم كعلاج مقارنة بالكنترول متوسطات سبارتيت ترانسفيريز (25.1 ± 5.7 وحدة/لتر)، النين ترانسفيريز (17 ± 4.2 وحدة/لتر)،الكالين فسفاتيز(189.3 ± 20.4 وحدة /لتر)) ولايوجد تغيير في متوسطات البروتين الكلي (7.3± 0.5) والألبومين (4± 0.3)عند مقارنتها بمجموعة الكنترول (متوسطات البروتين الكلي (74.6 ± 0.5) والألبومين (40.8 ± 0.3)

الخلاصة : خلصت الدراسة الي ان هنالك زيادة في نشاط إنزيمات الامينوترانسفيريز والالكالين فسفتيز وسط الأطفال السودانيين المصابين بالصرع .

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List of abbreviations

Abbreviations	Full text
ALT	Alanine aminotransferase
Alb	Albumin
ALP	Alkaline Phosphatase
AEDs	Anti epileptic drugs
AST	Aspartate aminotransferase
CLB	Clobazam
CBZ	Clobazam
CZP	Clonazepam
EEG	Electro encephalography
ESL	Eslicarbazepine
ETS	Ethosuximide
FBM	Felbamate
GPT	Gabapentin
GABA	Gama amino butyric acid
GGT	Gamma glutamyle trans peptidase
LCS	Lacosamide
LDH	Lactate dehydrogenase
LTG	Lamotrigine
LEV	Levetiracetam
LFT	Liver function test
LP	Lumber puncture
MRI	Magnetic resonance imaging
MDH	Malate dehydrogenase
NADH	Nicotine amide adenine dinucleotide
OXC	Oxcarbalin
PER	Perampanel
PB	Phenobarbital
PHT	Phenytoin
PGB	Pregabalin
RTG	Retigabine
RUF	Rufinamide
STP	Stiripentol
STM	Sulthiame
TPM	Topiramate
TP	Total protein
VPA	Valproic acid

Chapter One

Introduction, Rationale and Objectives

1. Introduction, Rationale, and Objectives

1.1 Introduction

Epilepsy is one of the oldest neurological conditions; it is characterized by recurrent, spontaneous brain seizures. Epilepsy is not contagious, although many underlying disease mechanism can lead to epilepsy, the cause of the disease is still unknown in about 50% of cases globally. The causes of epilepsy are divided to the following categories structural, genetic, infectious, metabolic, immune and unknown like, brain damage from prenatal or perinatal causes (e.g. loss of oxygen or trauma during birth, low birth weight), congenital abnormalities or genetic condition, stroke, infection of brain (meningitis, encephalitis), certain genetic syndrome, Brain tumor (Who., 2019).

About 70-80% of the patients who develop epilepsy may expect to have their seizures controlled with optimal antiepileptic therapy (Naithan et al., 2010). All anti-convulsion medications are associated with adverse effects which may have significantly impact on the quality of life and contributing to non compliance and, in rare circumstances be, potentially live threatening (Bengleil and Sherif., 2018).Valproate (VPA) is abroad spectrum anti-epileptic drug used for treatment of certain types of seizures since 1970, it can also used in other conditions including some psychiatric disorders and prophylaxis of migraine (Nanau and Neuman., 2013).although VPA is an old anti epileptic drug, it is still widely administered for epileptic patients, since it is favorably safe and in expensive (Jankovic and Dostic., 2012).VPA may cause transient and non hazardous side effects as well as serious and life threatening side effects. Non hazardous side effect includes weight gain, drowsiness, hair loss, tremor, nausea, headache and others (Gerstrert et al., 2007). Serious side effect include hepatotoxicity, encephalopathy, pancreatitis, bone marrow suppression, coagulation disorders (Verrotti et al., 2002).

Liver is the primary organ for drug metabolism and elimination for many anti epileptic drug (AED) and thus is subjected to drug induced toxicity. There is wide range of hepatotoxic reactions, from mild and transient elevation of hepatic enzymes to fatal hepatic failure (Hussein et al., 2013). Liver enzymes can service as markers of hepatocellular injury e.g. AST, ALT or of an obstruction in bile flow cholestasis e.g. ALP and GGT.

Although these enzymes are elevated in liver disease, the elevation can also be secondary to enzyme induction without hepatic damage (Ahmed and Siddiqi., 2006).

There are many substances capable of damaging the liver with several very different mechanisms, and there are many chemical agents that cause hepatotoxicity and these agents are called Hepatotoxins. These cause hepatotoxicity by the generation of free radicals and damage the liver cells and cause many liver diseases (Asija etal., 2014). The hepatotoxicity induced by anti epileptic drug occurs either because of production of reactive toxic metabolites or induction of immune allergic reactions (Hussein et al., 2013).

1.2 Rationale:

Epilepsy affecting about 50 million individuals worldwide and 90% of them are from developing countries (Kumar et al., 2012). Sodium Valproate is highly effective in control of Epilepsy especially absence seizure, myoclonic and atonic seizure. Sodium Valproate is usually well tolerated, but serious complications including hepatotoxicity and hyperammonemic encephalopathy may occur (Gamit et al., 2013). VPA has two hepatotoxic component, first is hypoglycin which leads to vomiting, sickness and micro vesicular liver steatosis ,second component is pantoic acid which inhibits β-oxidation and causes micro vesicular liver steatosis ,this process could be controlled by dose reduction in rare cases ,severe hepatic failure like ray syndrome can be happened,however, reversible increases in liver enzymes have been detected in 15-30% of VPA treated patient(Karaoglu et al.,2009).In Sudan there is no recent study about enzymes level and albumin among Sudanese children on sodium valproate treatment so that why we attempt this study.

1.3 Objectives:

1.3.1 General objective

To assess plasma aminotransferase (AST, ALT), and alkaline Phosphatase (ALP) activities among Sudanese children with epilepsy used sodium valproate as treatment.

1.3.2 Specific objectives:

1. To measure plasma ALT, AST, ALP activities, total protein and albumin concentrations in study group.
2. To compare between ALT, AST, ALP, total protein and albumin according to duration of disease, severity and sex.

Chapter Two

Literature Review

2. Literature Review

2.1 Epilepsy:

Epilepsy is derived from the Greek word "epilambanein" which means "to seize or attack from above". The belief held in many countries is that a person with epilepsy is possessed by supernatural forces or powers. This is largely responsible for the stigma against persons living with epilepsy. This widely held belief is incorrect as there is now evidence that seizures are the result of abnormal electrical discharges involving a group of brain cells (Kioy et al., 2016).

Epilepsy is 'a group of neurological disorders characterized by epileptic seizures (Fisher et al., 2014). An epileptic seizure is 'a transient occurrence of signs and or symptoms due to abnormal synchronous neuronal activity in the brain' (Fisher et al., 2005). According to the International League Against Epilepsy (ILAE) official report in 2014, epilepsy should be considered in any of the following conditions: two or more seizures occurring >24 hours apart, one unprovoked seizure and a probability of recurrent seizure risk over 60%, and diagnosis of an epilepsy syndrome (Fisher et al., 2014) . If there is any epileptiform discharge on electroencephalography, or a potential epileptogenic structure on brain imaging, the probability of recurrent epileptic seizure exceeds 60%. In addition, the ILAE official report in 2014 re-defined epilepsy as a disease, and suggested a concept of resolved epilepsy. Epilepsy is considered to be resolved if the epileptic is now past the applicable age, or has remained seizure-free over a decade despite a 5-year interruption of anticonvulsant treatment. However, 'resolved' and 'remission or cures' are not entirely equivalent (Kioy et al., 2016).Epilepsy is a disease of the brain characterized by enduring predisposition to generate epileptic seizures (Perucca., 2018) it is one of the most common neurological illnesses , affecting individuals of any age and ethnicity. In industrialized countries, 3-4 % of people will develop epilepsy during their lifetime ,the risk is higher in resource-poor countries (Beghi and Hesdorffer et al., 2010), epilepsy has deleterious effects on social, vocational, physical well-being, In the global burden of disease 2010 study, severe epilepsy ranked fourth among 220 health conditions in terms of disability weight (Salmon and Vos et al.,2014).

2.1.1Epidemiology of Epilepsy

Epilepsy knows no geographical, racial or social boundaries. It occurs in men and women at all ages, but is most frequently diagnosed in early childhood (70% are below the age of twenty years) and old age. Anyone can potentially develop seizures. In fact, up to 5% of the world's population may have a single seizure at some time in their lives, but only a proportion will have epilepsy as defined above (Kioy et al ., 2016).

The prevalence of a disorder is the proportion of a population with that disorder at a given point in time usually expressed per 1,000 populations. From many studies around the world it has been estimated that the mean prevalence of lifetime epilepsy is approximately 8.2 per 1,000 of the general population. It is likely that around 70 million people in the world have epilepsy at any one time. The lifetime prevalence of epilepsy (i.e. the number of people presently in the world who have epilepsy now or have had it in the past or will experience it in the future) is approximately 100 million people, This may, however, be an underestimate as some studies in developing countries suggest a prevalence of more than 10 per 1,000(Kioy et al., 2016)

The incidence of a disorder is the number of new cases reported within a given period of time usually expressed per 100,000 people in the year of observation. Studies indicate that the annual incidence of epilepsy ranges between 50 per 100,000 populations in developed countries to 82 per 100,000 populations in resource poor countries. This regional disparity in the incidence of epilepsy is attributed to the higher prevalence of risk factors or conditions which can lead to permanent brain damage. These conditions include brain trauma, meningitis, HIV/AIDS, cerebral malaria and pre and peri-natal complications, of the estimated 70 million people living with epilepsy in the world, nearly 50 million have no access to quality treatment and care (Kioy et al., 2016).

2 .1.2 Classifications of epilepsy and seizures:

Epileptic seizures are categorized by seizure onset into:

Partial (**Focal**)**seizures** "arising with in network limited to one hemisphere "include: Complex partial (impaired consciousness, typically frontal or temporal lobe ones, often stereotyped for Ind, average duration 1-3 minutes and Simple partial onset can be followed by impaired consciousness, fragmentally sex in adult ones epilepsy, automatism: involuntary movement.

Generalized seizures "arising at some point within, and rapidly engaging, bilaterally distributed networks" include: Absence is a sudden interruption of activities accompanied by a blank stare with occasional deviation of the eyes lasting a few seconds to half a minute with subsequent rapid recovery a typical types with other symptoms can also be found, Tonic is arrest of ventilation can cause cyanosis, an increase in muscle contraction, lasting from a few seconds to some minutes, Clonic is symmetrical or a symmetrical jerking of the same group of muscle, without tonic phase, Myoclonic is sudden, brief contractions of muscles resulting in dropping or spilling things and/or falls, may be localized or generalized.

Atonic is sudden loss of muscle tone lasting a few seconds who can affected the head, body, arms and legs and Tonic-clonic (Brodie et al., 2018).

Seizures of unknown onset"when there is insufficient information to classify to seizures focal or generalized (Perucca et al; 2018).

2.1.3 Causes of epilepsy

Epilepsy can have both genetic and acquired causes with interaction of these factors in many cases; established acquired causes include serious brain trauma, stroke, tumors and problems in the brain as a result of previous infections (Berkovic et al., 2006) In about 60% of cases the cause is unknown; epilepsies caused by genetic, congenital or developmental conditions are more common among younger people, while brain tumors and strokes are more likely in older people (Who., 2019).

2.1.4 Diagnosis of epilepsy

The diagnosis of epilepsy is made primarily on clinical grounds. Supporting investigations include electroencephalography (EEG) and neuroimaging, primarily magnetic resonance imaging (MRI). According to the 2014 practical definition of epilepsy of the international league against epilepsy, epilepsy can be diagnosed: After at least two unprovoked seizures more than 24 hours apart, After one unprovoked seizure when there is $\geq$ 60% chance of seizure recurrence over the next 10 years ; or When an epilepsy syndrome can be identified (Fisher and Acevedo et al., 2014).

Diagnosis includes clinical and medical history carefully taken medical history from the patient and eyewitness is the most important step in the diagnosis of the patient with repeated seizures. Detailed seizure history and current episode (ictal, preictal, postictal,

onset, frequency, time, triggers, and seizure characteristics), Physical examination begins with observation (presence of weakness or spasticity), the behavior and ability to communicate. Focal deficits, Todd's paralysis. Laboratory investigations: Full heamogram, serum electrolytes (sodium, calcium, potassium, magnesium), Bloodsugar, liver function tests, screening for infections (HIV/ Malaria),Cerebrospinal fluid examination LP if fever or meningeal signs present ,Chest radiograph, CT brain scan (Kioy et al., 2016).

2.1.5 Path physiology of epilepsy:

Seizures are paroxysmal manifestations of the cerebral cortex. a seizure results when a sudden imbalance occurs between the excitatory and inhibitory forces within the network of cortical neurons. The basic physiology of a seizure episode is detected to in an unstable cell membrane or it is surrounding/adjacent supportive cells. The seizure originates from the grey matter of cortical or subcortical area (Hirtz et al., 2007). Initially a small number of neurons fire abnormally. normal membrane conductance and inhibitory synaptic current breakdown and excess excitability spread either locally to produce a focal seizure or more widely to produce a generalized seizure .this onset propagates by physiologic pathways to involve adjacent to remote areas .as abnormality of potassium conductance, a defect in the voltage activated ion channels, or a deficiency in the membrane ATPase linked to ion transport may cause neuronal membrane unstable and cause a seizure. Certain neurotransmitters (e.g. glutamate, Aspartate, acetyl choline, norepinephrine) enhance the excitability and propagation of neurons; activity, whereas gamma amino butyric acid (GABA) and dopamine inhibit neuronal activity and propagation. During a seizure, the demand for blood flow to the brain increases to carry off co2 and to bring substrate for metabolic activity of the neurons, as the seizure prolongs, the suffers more from ischemia that may result in neuronal destruction and brain damage. Mutation In several genes may be linked to some types of epilepsy. Genes that code for protein subunits of voltage-sensitive and ligand –activated ion channels have been associated with the generalized epilepsy and infantile seizure syndrome. one speculated mechanism for some forms of inherited epilepsy are mutation of the genes which code for sodium channels proteins these defective sodium's channels remain open for long time and causing the neurons hyper excitable as a result glutamate an excitatory neurotransmitter may be released in large amount form the neurons which by binding with nearby glutamatergic neurons triggers

excessive calcium release in the post synaptic cells which may be neurotoxin to the affected cells (Meisler et al., 2005).

2.2 Anti-Epileptic Drugs (AEDs):

Anticonvulsants (also commonly known as antiepileptic drugs or as anti-seizure drugs) are a diverse group of pharmacological agents used in treatment of epileptic seizures. Anticonvulsants are also increasingly being used in the treatment of bipolar disorder, and borderline personality disorder, since many seem to act as mood stabilizers, and for the treatment of neuropathic pain, anticonvulsants suppress the excessive rapid firing of neurons during seizures, Anticonvulsants also prevent the spread of the seizure within the brain (Rogawski et al., 2004)

The goal of anti-epileptic drug treatment is optimize seizure control and quality of life while minimizing treatment toxicity(Yoshimura etal.,2019) appropriate pharmacological management can result in freedom from seizures in 60-70% of patients ,with more than 90% of them being controlled by mono therapy (Yoshimura etal.,2019).AEDs are associated with 8.3% of reports of drug induce liver injury ,which is major reasons for their withdrawal from therapeutic use (Bjornsson .,2008) anti-epileptic drugs includes first-generation AEDs [carbamazepine (CBZ)clobazam (CLB), clonazepam (CZP), ethosuximide (ETS), Phenobarbital (PB), phenytoin (PHT), sulthiame (STM), valproic acid (VPA)] and second-generation AEDs [felbamate (FBM), gabapentin (GPT), lamotrigine (LTG), levetiracetam (LEV), oxcarbazepine (OXC), pregabalin (PGB), tiagabine (TGB), topiramate (TPM), vigabatrin (GVG), zonisamide (ZNS)] (Rosati etal., 2015). The most recently approved drugs, referred to as third-generation or newer AEDs, include eslicarbazepine acetate (ESL), lacosamide (LCS), perampanel (PER), retigabine (RTG), rufinamide (RUF), and stiripentol (STP). Most of the second- and third-generation AEDs are licensed as an adjunctive treatment of epilepsy in adults, and are therefore used off-label in pediatric populationson the basis of increasing evidence of their potential efficacy in children, especially in those older than 12 years of age (Rosati etal., 2015).In our study concentrated on children treated with sodium valproate according to availability of specimen.

Valproate (VPA) is an antiepileptic drug that is used in neurology for the treatment of generalized and partial epilepsy, especially in cases in which absence, myoclonic, tonic-

clonic, atonic, and mixed type seizures are seen, and for prophylaxis of childhood febrile convulsions (Wheless et al., 2007). It is also preferred in cases in which the type of epileptic seizure cannot be determined and for prophylaxis of migraine headaches (Admir and Akdeniz., 2009). In psychiatry it is used for the treatment of bipolar disorder manic episodes and for prophylactic treatment, it can also be used long-term as a supplemental agent for impulse control disorder and schizophrenic disorder (Admir and Akdeniz., 2009). The most commonly encountered side effects of VPA treatment are associated with the gastrointestinal system, including nausea, vomiting, and diarrhea. Other common side effects are sedation, ataxia, dysarthria, and tremor. Side effects like hair loss, weight gain, changes in liver function tests, hyperammonemia, and benign thrombocytopenia have also been reported. One of the side effects that can be observed, but is not frequently considered, is the negative effect on reproductive functions (Bailer and Yagen., 2007).

Sodium valproate was first marketed as an anti convulsant almost50 years ago in France, it is indications have expanded and it is now the most prescribed anti-epileptic drug worldwide (Gill.etal., 2011).

2.2.1 Mechanism of action of sodium valproate:

Previously, there was evidence suggesting that VPA has participatory role in increasing γ.amino butyric acid (GABA) concentration with in the brain via multiple enzyme systems (GABA Transaminase, α –ketoglutarate dehydrogenase and succinic semi aldehye dehydrogenase)(kammerer et al., 2011).however, there has been conflicting evidence indicating that concentration that are therapeutically effective have shown to not affect these enzyme systems .thus, supporting the theory that many undefined mechanisms contribute to it is therapeutic effect ,possibly including reduction in excitatory neurotransmission, modification of monoamines (Marland ,Nordeng and Gundersen) or weak inhibition of voltage gated sodium channels (Large et al., 2009).

2.2.2 Pharmacology of sodium valproate:

Valproate is available in tablet, syrup and intravenous formulation there is no single mechanism of action that can explain valproate broad effects on neural tissue it is pharmacological effects include increased gamma-amino butyric acid transmission, reduced release of excitatory amino acids, blocked of voltage –gated sodium channels, and Modulation of dopaminergic and serotoergic transmission (zawab and carmody., 2014)

When fasting oral valproate is rapidly absorbed and reaches peak plasma concentration within4-7 hours. It is highly plasma protein bound and has a half-life of 8-20 hours in most patients ,but this may occasionally be much longer (linde etal ., 2013).

Relationship between dose, plasma concentration and therapeutic effect is not understood .valproate is almost completely metabolized in liver, mainly by glucuronidation. It then undergoes further metabolites which is complex and involves several cytocrome p450 enzyme systems. It has multiple metabolites which may contribute to both it is efficacy and toxicity (zawab and carmody., 2014)

Valproate (VPA) has very complicated biotransformation; hepatic biotransformation formed is the main route of elimination and involves glucuronization, β-oxidation and cyp450 mediated oxidation 30%-50% of VPA may be metabolized via glucuronization, 30% of VPA metabolism occurs by β-oxidation in the mitochondrial and 10% of VPA is biotransformation through CYP450 mediated oxidation (lee et al., 2013).

Moreover, VPA can inhibit histone deacetylase (HDAC), which is crucial factor in the pathogenesis of cancer and transcription regulation. VPA as well as other (HDAC) inhibitors is able to alter expression of many genes involved in the modulation of cell growth, differentiation and apoptosis (yilmaz et al., 2014).

2.2.3 Contraindication of sodium valproate:

The toxicity of VPA was low, with minor dose-related side effects (weight gain, hair loss, nausea and vomiting (liyond., 2013) and rarity idiosyncratic reaction(hematological toxicity, hepatotoxicity, pancreatitis and polycystic ovary syndrome (Zaccara, Franciotta and Peruca., 2007).These are usually of higher incidence when VPA is used in poly therapy and are significantly more common in pediatric administration (Chung et al., 2011). however ,VPA is a known human teratogen (cause birth defects)(Holmes., 2011) with high contraindications when administered during the first trimester of pregnancy (Lioyd., 2013).

2.3 The liver

The liver is vital organ it has a wide range of functions, including of biochemical necessary for digestion, synthesis, detoxification, storage and other functions (Earthert., 2015) Several biochemical Tests are useful in the evaluation and management of patients with hepatic dysfunction, these tests can be used to detect the presence of liver disease,

distinguish among different types of liver disorders, evaluate liver damage and follow the response to treatment(Clatchy., 2002).

The liver has central and critical biochemical role in metabolism, digestion, detoxification and elimination of substances from the body. All blood from the intestinal tract initially passes through the liver where product derived from digestion of food are processed transformed and stored .the liver metabolized both endogenous and exogenous compound such as drugs and toxin through biotransformation, allowing their elimination (Burtis et al., 2008).

2.4 Liver function test (LFT)

The liver function test (LFT) is group of blood tests that give information about the state of a patient liver .This testing is performed on a patient blood sample ,some tests are associated with functionality (albumin),some with cellular integrity (transaminases) and some with conditions linked to biliary tract (gamma glutamyle transferase and alkaline Phosphatase)(Lee., 2009)LFT is often given to screen for liver infections such as hepatitis c, to monitor the side effect of certain medication s, disease of liver and in symptoms of liver disorder ,commonly used tests to check liver functions are alanine aminotransferase(ALT),Aspartate aminotransferase(AST),Albumin, and bilirubin .ALT,AST test enzymes that liver releases in response to damage or disease (Burtis et al ., 2008).

2.4.1. Alanine Aminotransferase (ALT)

Is transferase with enzymatic activity, it catalyzes the transfer of an amino group from alanine to alpha-ketoglutarate with the formation of glutamate and pyruvate. ALT distributed in many tissues, with comparatively high concentrations in the liver. It is considered the more liver specific enzyme of transferase (Bishop et al., 2010).Clinical applications of ALT assays are confined mainly to evaluations of hepatic disorders. Higher elevations are found in hepatocellular disorders than extra hepatic or intrahepatic obstructive disorder. in acute inflammatory conditions of the liver ,ALT elevations are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half –life of ALT in serum (16and 24, respectively)(Bishop et al., 2010).

2.4.2 Aspartate Aminotransferase (AST)

AST is an enzyme found in the liver and heart, but it is also found in many other tissues, including muscle. Red blood cell, pancreas, kidney and brain. Damage to these organs or hemolysis releases the enzyme; resulting in elevated AST levels in serum .serum levels generally parallel the extent of damage (Bishop et al., 2010)

Conditions associated with very high AST levels more than 10 times than highest normal value in case of liver damage and tumor necrosis, moderately high AST are seen in liver disease, heart disease, chronic kidney damage, muscle injury and hemolysis, on the hand slightly high AST levels are seen in fatty changes in liver, alcohol abuse, mononucleosis and drugs (Bishop et al., 2010).

2.4.3Alkaline Phosphatase (ALP)

Is hydrolase enzyme responsible for removing phosphate groups from many type of molecules, including nucleotide, proteins and alkaloid. ALP is an important enzyme mainly derived from the liver, bones and leukocytes. Increase in ALP level in serum is frequently associated with variety of disease, Physiological increases are found during bone growth while pathological increase largely associated with hepatobiliary and bone disease (Shaheen et al., 2009).

2.4.4 Total protein

Variation in plasma protein concentrations can be due to changes in any of three factors: the rate of protein synthesis, the rate of removal and the volume of distribution. The concentration of proteins in plasma is affected by posture: an increase in concentraion10-20% occurs within 30 min of becoming up-right after a period of recumbence. Also, if a tourniquet is applied before vene puncture, a significant rise in protein concentration can occur within a few minutes .in both cases, the change in protein concentration is caused by increase diffusion of fluid from the vascular in to the interstitial compartment .only changes in the more abundant protein (I.e. albumin or immunoglobulin) will have a significant effect on the total protein concentration. The total protein concentration can also fall rapidly if capillary permeability increases, because protein will diffuse out in to the interstitial space. This can be seen, for example, in patients with septicemia or generalized inflammatory conditions (Marshall et al., 2012).

2.4.5 Albumin

Albumin is synthesized in liver and it is concentration in the plasma is in part a reflection of the functional capacity of the organs. Plasma albumin concentration tends to decrease in chronic liver disease (Marshall et al., 2012). The most a abundant protein in serum (3.5-5g/dl, which constitutes approximately half of all serum proteins), albumin has several important characteristic. It is an ionic protein, containing and a abundance of Aspartate and glutamate residues; among all serum proteins, it has mild range molecular weight 67kd; and it has longer than average half-life of approximately 20 days .albumin help maintain osmotic balance between intravascular and interstitial space .albumin also functions as a transport protein for calcium, unconjugated bilirubin, thyroid hormone, and many drugs (Marshall et al., 2012). Albumin synthesis is an important function of the liver approximately 10g is synthesized and secreted daily, with progressive liver disease serum albumin level fall, reflecting decreased synthesis. Albumin levels are dependent on a number of other factors such as the nutritional status, catabolism, hormonal factors, and urinary and gastrointestinal losses (Limdei and Hyde., 2003).

2.5 Relation between anti-epileptic sodium valproate and liver

The liver is the primary organ for drug metabolism and elimination for many anti-epileptic drugs and thus is subjected to drug-induced toxicity. There is a wide range of hepatotoxic reactions, from mild and transient elevations of hepatic enzymes to fatal hepatic failure .liver enzymes can serve as markers of hepatocellular injury e.g.ATL, AST or of an obstruction in bile cholestasis e.g. alkaline Phosphatase (ALP) and gamma glutamyle transferase (GGT (George., 2016).

Hepatotoxicity due to VPA appears in two forms, the benign form exists as a mild dose-related elevation of liver enzymes that appears with reduction in dosage or discontinuation of drug. The other form is a less common non dose-related disorder characterized by hepatic failure ,the mechanism of VPA hepatotoxicity is thought to be due to mitochondrial toxicity, may be from inhibition of β.oxidation and subsequent loss of mitochondrial function, transaminitis is elevation of transaminases in the liver, most commonly ALT and AST . These enzymes are normally present in high concentration within the hepatocytes, The presence of elevated serum concentration of one or more of these enzymes suggest hepatocytes have lysed in response to some noxious insult, resulting the cell contents to

circulate in the blood (Alderman., 2012) . other causes of transaminitis include alcoholic liver disease, viral hepatitis and others; in case of drug induced transaminitis ,the levels of transaminases usually come down after the offending drug is stopped (Nampoothiri & Askshm.,2015).

Chapter Three

Materials and Methods

3. Materials and methods

3.1 Materials

3.1.1 Study design, area and duration

This was a comparative cross-sectional hospital-based study. This was conducted in Khartoum state in Mohammed Elamin Hammed Pediatric Hospital during March to July 2019.

3.1.2 Study population

Forty Sudanese children with epilepsy were enrolled in this study as attest group and forty apparently healthy children were include as control group ,both the control and test group were matched for age and sex.

3.1.2.1 Inclusion criteria

Sudanese Patients diagnosed with epilepsy on sodium valproate treatment.

3.1.2.2 Exclusion criteria

Patients with liver disease, Bone disease, Muscle disease, renal disease and Malnutrition, Were excluded from this study.

3.1.3 Ethical Consideration

Permission of this study was obtained from

Local authorities in the area of the study

The objectives of the study were explained to all individuals participating in the study

Verbal informed consent was obtained from each participant in the study

3.1.4 Data Collection

Interview with the test group and controls were done to obtain the clinical data and Questionnaire (see appendix 1 page) was especially designed to obtain information which help in either including or excluding certain individual in or from the study.

3.1.5 Blood Sampling

After informed consent and use of local antiseptic for skin (70% ethanol), 3 ml of venous blood was collected from each participant in this study, using sterile disposable plastic syringes, the blood was collected from the cubital vein and centrifuged for 5 minutes at 3000 rpm to obtain plasma and then plasma was kept at-20 ºC till the time of analysis.

3.2 Methodology

3.2.1 Measurement of plasma level of Alanine aminotransferase (ALT/SGPT)

Principle

ALT was measured by used UV assay according to international federation of clinical chemistry and laboratory method without pyrodoxal.

α- oxoglutarate + L-Alanine $\xrightarrow{ALT}$ L-glutamate + pyruvate

Pyruvate + NAÑDH+H $\xrightarrow{LDH}$ L-lactate + NAD + H2O (Schumann et al., 2002).

Reagent composition and procedure of alanine aminotransferase (see appendix 2page).

3.2.2Measurement of level of plasma Aspartate aminotransferase (AST/SGOT)

Principle

L-Aspartate + α-Oxoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-glutamate

Oxaloacetate +NADH+H $\xrightarrow{MDH}$ L-malate + L-malate +NAD+H2O (Schumann et al., 2002).

Reagent composition and procedure of Aspartate aminotransferase (see appendix 3 page).

3.2.3 Measurement of level plasma of Alkaline Phosphatase (ALP)

Principle

P-Nitro phenyl phosphate + H_2O $\xrightarrow{ALP/Mg2+}$ P-Nitro phenol+ p_i(Moss and Henderson., 1999).

Reagent composition and procedure of alkaline Phosphatase (see appendix 4page).

3.2.4 Measurement of plasma level of Total protein

Principle

Biuret method at alkaline solution copper ions combined with protein to produce a blue violet color complex. The absorbency increase is directly proportional to the concentration of protein (Thomas., 1998).

Reagent composition and procedure of total protein (see appendix 5 page).

3.2.5 Measurement of plasma level of Albumin

Principle

Bromocresol green (BCG) method at slightly acid PH (PH), serum albumin combines with Bromocresol green to produce a glaucous complex. The absorbency increase is directly proportional to the concentration of albumin (Thomas., 1998).

Reagent composition and procedure of Albumin (see appendix6 page).

3.3 Quality Control

The precision and accuracy of the method used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

3.4 Statistical analysis

The data obtained from all participants were recorded and analyzed by using statistical package for social science (SPSS) software version20. the result were expressed as means ± SD or in count and percentage ,t-test was used to find out the difference between means of compared group, for all statistical comparisons a p-value of ≤ 0.05 was considered statistical significant.

Chapter Four

Results

4. Results

A total of 40 Sudanese children with epilepsy in Khartoum State Sudan were taken as test group, compared to 40 apparently health children were taken as control group.

The results obtained were illustrated as follow:

Table (4-1) shows demographic characteristic of epileptic patients

Table (4-2) shows significant increase in AST, ALT, ALP activities (Mean ± SD $p \leq 0.05$), and insignificant different in TP & Alb (Mean ±SD, $p \geq 0.05$), between epileptic patient compare to control group.

Table (4-3) shows insignificant difference in AST, ALT, ALP, TP, Alb ($p \geq 0.05$) in boys group versus girls group.

Table (4-4) shows insignificant difference in study parameters in the duration of disease.

Table (4-5) shows insignificant difference in study parameters across the severity of convulsion.

Table (4-1) The Demographic Characteristics of Cases

Characteristic	**Percentage**
Family History	
Yes	18%
No	82%
Severity of Disease	
Severe	72%
Moderate	28%
Duration of Disease	
0- 3 year	77%
≥ 4 year	23%

Table (4-2) Means comparison of study parameters in case versus control group

Parameters	**Case (Mean ±SD)**	**Control (Mean ±SD)**	***P-value***
ASTU/L	30.4±12.1	25.1±5.7	0.01*
ALT U/L	21.5±12.1	17.0±4.2	0.03*
ALP U/L	232.8±103.9	189.3±20.4	0.01*
TPg/L	72.9±0.6	74.6±0.5	0.15
ALBg/L	39.9±0.3	40.8±0.3	0.22

The table shows the mean ±standard deviation and probability (p)

Independent t-test used for comparison

P-value ≤ 0.05 is considered significant.

Table (4-3) Means comparison of study parameters in boys group versus girls group

Parameters	**Boys (Mean ±SD)**	**Girls (Mean ±SD)**	***P-value***
AST U/L	32.7±13.5	27.3±9.3	0.17
ALT U/L	23.0±13.5	19.5±9.99	0.37
ALP U/L	235.1±106.7	229.6±103.2	0.87
TP g/L	73.0±0.6	72.7±0.5	0.87
ALB g/L	39.6±0.3	40.4±0.3	0.47

The table shows the mean ±standard deviation and probability (p)

Independent t-test used for comparison

P-value ≤ 0.05 is considered significant.

Table (4-4) Means comparison of study parameters across the duration of disease

Parameters	**0-3 Year(Mean±SD)**	**≥4 Years (Mean±SD)**	***P-value***
AST U/L	29.0±11.7	35.11±12.8	0.19
ALTU/L	19.7±11.4	27.67±13.2	0.08
ALP U/L	243.9±104	194.7±99.7	0.22
TP g/L	72.4±0.6	74.7±0.5	0.28
ALB g/L	39.8±0.4	40.6±0.30	0.55

The table shows the mean ±standard deviation and probability (p)

Independent t-test used for comparison

P-value ≤ 0.05 is considered significant.

Table (4-5) Means comparison of study parameters across the severity of convulsion

Parameters	**Severe (Mean ±SD)(n=29)**	**Moderate (Mean ± SD)(n=11)**	***P-value***
AST U/L	31.3±12.6	27.9±10.7	0.43
ALT U/L	21.3±12.1	22.1±12.8	0.85
ALP U/L	232.1±113.2	234.5±79.3	0.95
TP g/L	72.8±0.6	73.1±0.6	0.83
ALB g/L	39.8±0.4	40.4±0.3	0.64

The table shows the mean ±standard deviation and probability (p)

Independent t-test used for comparison

P-value ≤ 0.05 is considered significan

Chapter Five

Discussion, Conclusion and Recommendations

5. Discussion, Conclusion and Recommendations

5.1 Discussion

Sodium valproate is a widely used and well tolerable drug in epileptic pediatric patient, VPA has many adverse effects which may be dose related or not, it has been associated with acute, rarely fatal hepatotoxicity (Karaoglu et al., 2009). VPA is commonly associated with mild elevations of liver enzymes, these elevations are usually transitory or dose related and not appears to be associated with hepatocellular injury (Hussein et al., 2013).This study was focused mainly on the estimation of liver parameters in epileptic children used sodium valproate.

The finding of this study was showed that the mean values of plasma ALT, AST activities in epileptic children were statistically significant increase when compared with control group ($p \leq 0.05$) ,this results agree with (Alemami et al., 2013,Gamit et al., 2013) ,whom found that the liver enzymes (AST,ALT) Activities ($p \leq 0.05$) significantly increased when compared with control, the increase in AST activity may be explained based on the fact that the AST is distributed in many other organs beside liver, muscle contain appreciable amounts of the AST, it is well known that anti-epileptic drug affect muscle causing their relaxation, which may be the cause of elevated activity of AST in patient (Elmassri et al ., 2013), also an increase in ALT activity could be explained on the bases that the patient administered high dose of drug over long period of time. Our study showed increase in plasma ALP activity in epileptic children when compared with control group (($p \leq 0.05$) similar results were also reported by (Elmassri et al., 2013, Salehiomran et al., 2010), because increased levels of ALP enzyme may reflect enzyme induction rather than lesions of the cells (Yushimura et al., 2019), or may be due the patients intake high dose of drug. On the other hand the means value of plasma total protein and albumin in epileptic children were statistically insignificant when compared with control group(($p \geq 0.05$) this result agree with study carried by(Faruk et al., 2007),reported that no statistical significant in total protein and albumin when compared with control ($p \geq 0.05$) , also agree with (attilakos et al., 2007), whom found that no statistical significant in total protein and significantly decreased in albumin, this may be due to administered large dose of valproate; the

valproate may directly or indirectly inhibit protein synthesis by interfering with urea cycle resulting alteration albumin synthesis(Ringo et al.,2003).

According to the sex(boys, girls) there were no statistical significant change in means of plasma Aspartate aminotransferase, alanine aminotransferase, alkaline Phosphatase activities ,and means of plasma total protein and albumin in children with epilepsy on sodium valproate treatment this results agree with (Catebusic et al., 2017) reported that there was no statistical significant between boys and girls.

5.2 Conclusion

Epileptic Sudanese children on sodium valproate treatment had increased levels of AST, ALT, ALP Activities, and normal level of total protein and Albumin in this study.

5.3 Recommendations:

1. Periodic monitoring of liver function test to detect early deterioration of liver function test.
2. Further research with other study design Cohort study.

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Appendix (1)

Sudan University of Science and Technology

College Of Graduate Studies

Master of Medical Laboratory Science

The assessment of liver parameters in Sudanese children used sodium Valproate

Patient Questionnaire

NO ( )

A. General Information:

1. Name:

2. Gender:

Male ( ) female ( )

B .Clinical Information:

1. Type of convulsion:

a. Complete ( ) b- partial ( )

2. Family History:

a. Yes ( )

1. First degree ( ) 2- second degree ( )

B. No ( )

3. Frequency of convulsion:

1. Mild ( ) 2. Moderate ( )

3.severe ( )

4. Duration of disease:

1. Months ( ) 2.Years ( )

5. Type of Treatment:

1. 2.

C. laboratory Investigation:

Specimen	Test	Result
Serum	AST	……U/L
Serum	ALT	……U/L
Serum	ALP	……U/L
Serum	TP	……g/L
Serum	ALB	……g/L

Appendix (2)

Alanine aminotransferase (ALT)

Reagents Component:

R1 Contain Tris buffer +L-alanine +LDH+NADH

R2 contain α-oxoglutrate +NADH

Assay Procedure

	Blank	**Sample**
Reagent 1 Dis water Sample Mixed, incubated for	1000µl 100µl _ 5Minute then added	1000µl _ 100µl
Reagent 2	250µl	250µl

Mixed thoroughly, read the absorbance after 1min and monitored time .read the absorbance again for additional 3 min

ΔA/min=(ΔA/min sample)-(ΔA/min blank)

Reference intervals

Male ≤41

Female ≤40

Appendix (3)

Aspartate aminotransferase (AST)

Reagents Component:

R1 Contain Tris buffer +L-Aspartate +LDH+ MDH+NADH

R2 contain α-oxoglutrate +NADH

Assay Procedure

	Blank	Sample
Reagent 1 Dis water Sample Mixed, incubated for	1000µl 100µl _ 5Minute then added	1000µl _ 100µl
Reagent 2	250µl	250µl

Mixed thoroughly, read the absorbance after 1min and monitored time .read the absorbance again for additional 3 min

ΔA/min= (ΔA/min sample)-(ΔA/min blank)

Reference intervals

Male ≤41U/L

Female ≤40U/L

Appendix (4)

Alkaline Phosphatase (ALP)

Reagent Component:

R1 contain AMP buffer +Magnesium acetate +zinc sulfate

R2 contain p-Nitro phenyl phosphate

Assay Procedure

	Blank	**Sample**
Reagent 1 Dis water Sample Mixed, incubated for	1000µl 20µl _ 2Minute at 37° C, then added	1000µl _ 20µl
Reagent 2	250µl	250µl

Mixed thoroughly, incubated at 37°C for 1min and then read the absorbance changed value within 3 min.

ΔA/min= (ΔA/min sample)-(ΔA/min blank)

Reference intervals

Reaction temperature	**Men**	**Women**
25ºC,up to	75UL=1.25µKat\L	68UL=1.13µKat\L
30ºC,up to	87UL=1.45µKat\L	80UL=1.33µKat\L
37ºC,up to	115U/l=1.92µKat/L	105U/L=1.75µKat/L

Appendix (5)

Total protein (TP)

Reagent component:

R contain sodium-potassium tartrate +sodium hydroxide +potassium iodide +cupric sulfate

Assay Procedure

	Blank	Sample
Reagent Dis water Sample	1000µl 20µl _	1000µl _ 20µl

Mixed thoroughly at 37°c, and read the absorbance10 min. Later

ΔA= (ΔA sample)-(ΔA blank)

Reference Intervals

Adults 66-83 g/l

Premature 57-80 g/l

Newborns 41-63 g/l

Appendix (6)

Albumin (ALB)

Reagent component

R contain Citrate buffer +Bromocresol green +Surfactant

Assay Procedure

	Blank	Sample
Reagent Dis water Sample	1000µl 20µl _	1000µl _ 20µl

Mixed thoroughly at 37°c, and read the absorbance10 min. Later

ΔA= (ΔA sample)-(ΔA blank)

Reference Intervals

3.5-5.5 g/dl