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Role of SCN1A and UGT2B7 Genetic Polymorphisms on The Response to Valproic acid Among Iraqi Epileptic Patients

A Thesis

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By

Ayat Abd Ul-Khaleq Jwad

B.Sc. in Pharmacy, University of AL-Mustansiriya

Supervised by

Asst. Prof. Dr

Ahmed Haqi Ismael

PhD. Pharmacology and Therapeutics

Asst. Prof. Dr

Alaa Abd -Alhasan Hamdan

PhD. Biotechnology

2022AD

1443AH

بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ}

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من الآية الحادي عشر

Supervisor Certification

We certify that this thesis was prepared by **(Ayat Abd ul-Khaleq Jwad)** under our supervision at the Department of Pharmacology and Toxicology at the College of Pharmacy / University of Karbala, as a partial requirement for the Master degree in Pharmacology and Toxicology

Supervisor:

Asst. Professor

Dr. Ahmed Haqi

Ismael

College of Pharmacy /

University of Karbala

Date: / / 2022

Supervisor:

Asst. Professor

Alaa Abd –Alhasan

Hamdan

College of Pharmacy /

University of Karbala

Date: / / 2022

In the view of the available recommendation, we forward the present thesis for debate by the examining committee.

Assist. Prof. Amal umran mosa

Head of Pharmacology and Toxicology Department

University of Karbala / College of Pharmacy

Committee Certification

We, the examining committee, certify that we have read the thesis; and have examined the student (**Ayat Abd ul-Khaleq Jwad**) in its contents, find it adequate as a thesis for the degree of Master in Pharmacology and Toxicology.

Thesis Committee Names:

Chairman

Asst. Prof. Dr. Ihsan S. Rabea

College of Pharmacy/ University of Kufa

Member

Asst. Prof.

Dr. Uday Abdul- Reda Hussein

College of Pharmacy/ University of AL- Ameed

Member

Asst. Prof.

Dr. Suzanne Jubair Abbas

College of Pharmacy/ University of Karbala

Supervisor

Asst. Prof.

Dr. Ahmed Haqi Ismael

College of Pharmacy/ University of Karbala

Supervisor

Asst. Prof.

Dr. Alaa Abd- Alhasan Hamdan

College of Pharmacy/ University of Karbala

Approved by

College of Pharmacy / University of Karbala

As a thesis for the degree of

Master in Pharmacology and Toxicology

Professor

Dr. Ahmed Salih Sahib

Dean

College of Pharmacy / University of Karbala

Seal

Higher Studies Registration

College of Pharmacy / University of Karbala

Dedication

To

all my teachers at all my grade levels

To

the source of giving and hope... My dear father

To

my greatest source of inspiration... My kind mother

To

My best support in all my life ... My husband (Ph. Mohammed Read) for his constant encouragement and support me during my study and research

To

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List of abbreviations

Abbreviations	Full-Text
AC	Adjusted concentration
AED	Antiepileptic drug
ALT	Alanine Aminotransferase
AST	Aspartate aminotransferase
ARMS-PCR	Amplification Refractory Mutation System-Polymerase Chain Reaction
AS-PCR	Allele Specific- Polymerase Chain Reaction
CMIA	Chemiluminescent Microparticle Immunoassay
DNA	Deoxyribonucleic Acid
EDTA	Ethylene diamine tetracetate
NADH	Nicotinamide adenine dinucleotide
PCR	Polymerase chain reaction
RLUs	Relative light units
SD	Standard deviation
SCN1A	Sodium voltage- gated channel alpha subunit 1
SNPs	Single nucleotide polymorphisms
TBE	Tris-borate EDTA
TDM	Therapeutic drug monitoring
UGT	Uridine diphosphate glucuronosyltransferase enzyme
UGT2B7	Uridine diphosphate glucuronosyltransferase family2 member B7
VPA	Valproic acid

List of normal laboratory values

Parameters	***Normal value***
Serum valproic acid concentration	50-100 (µg/ml)
Serum valproic acid Adjusted Concentration (AC)	5-6.66 (µg/mL per mg/kg)
Serum Total bilirubin concentration	0.1-1.2 mg/dl
Serum Alanine Aminotransferase concentration (ALT)	0-40 U/L
Serum Aspartate aminotransferase (AST) concentration	UP to 40 U/L

Abstract

Background: Sodium-channel blocking with valproic acid (VPA) is widly used in epileptic adult and pediatric patients, the Nav1.1 channel subtypes are the major sodium channels in the central nervous system encoded by SCN1A gene. SCN1A mutations most frequently associated to a wide variety of severities hereditary epilepsy and may be major factors of individual phenotypic variances in response to AEDs. The efficacy of valproic acid (VPA) varies greatly in clinical treatment of epileptic patients has been linked to polymorphisms in genes involved in valproic acid site of action. This led to believe that the SCN1A genotype may play a role in valproic acid responsive. the second factor which is VPA steady-state target site concentration is influenced by genetic differences in its absorption, transport, and metabolism .UGT2B7 is a key enzyme in the VPA metabolism, UGT2B7 functional mutations may be one predictor of individual variability in VPA pharmacokinetics, and VPA dose in individuals should be adjusted to the therapeutic range to assure achievement and to avoid VPA adverse drug reactions such as hepatotoxicity in high-risk patients. In this study two single-nucleotide polymorphisms (SNPs) in two potential genes related to receptor site of action and drug-metabolizing enzyme were genotyped.

Aims of the study: Study the effect of genetic polymorphism in SCN1A (rs2298771) and UGT2B7 (rs7439366) genes with VPA response via determinant variability in VPA pharmacodynamics and pharmacokinetics in the Karbala province epileptic patients. Furthermore, this study investigated the effect of the UGT2B7 (rs7439366) polymorphisms on serum liver enzyme level

to estimate genetic risk factors for VPA-induced hepatotoxicity in the Karbala province epileptic population.

Subjects and Methods: This study was performed at the Neurology consultancy at Imam AL-Hussein Medical City / Karbala –Iraq and in the out clinic patients with newly diagnosed epilepsy and other with previously AED failure treatment. patients are chosen from October 2020 to June 2021 while seeking medical care, they are chosen depending on their medical history, clinical manifestations of epilepsy and dependence on the International League Against Epilepsy's diagnostic criteria (ILAE). It is a prospective study done on one hundred thirteen patients with epilepsy were start receiving valproic acid monothrrapy 10-15 mg/kg from the begining of the study to six months duration. Clinical data were collected monthly for each patient kept a recorded of the date, number of seizure attacks on a daily record card, re-evaluation processes were done at the third and sixth month after enrolment. 3-month retrospective baseline was used to establish the baseline seizure frequency. Blood samples were collected from each patient who had given consent for genetic testing and measurement of serum valproic acid concentration at steady state condition after three and six months, and liver enzymes test (AST), (ALT), (Total Bilirubin) before and after six months of treatments .

Results: In this study there was statistically significant association of SCN1A rs2298771 c.3184 A/G polymorphism with the differences in clinical parameters and with VPA response and there was statistically significant association of UGT2B7 (rs7439366) c.802 C/T polymorphism with the differences in serum VPA concentration and statistically association with many liver enzymes as adverse effect, while this SNP had a non-significant association with valproic acid response among epileptic patients.

Conclusion: Genetic polymorphism of SCN1A (rs2298771) c.3184 A/G is associated with an increase in the incidence of valproic acid resistance in G mutant allele carriers and UGT2B7 (rs7439366) c.802 C/T polymorphisms may influence VPA metabolism and may be an important determinant individual VPA concentration in T allele mutant carriers . Furthermore determining UGT2B7 (rs7439366) c.802 C/T polymorphisms shows that people with the CC genotype should be monitored more closely for the possibility of hepatotoxicity.

Chapter one

Introduction

1.1. Epilepsy

Epilepsy is considered a central nervous system disorder in which nerve cell activity in the brain becomes disturbed and causing a recurrent, unprovoked seizure revealed by an abnormal and extreme corresponding discharge of a set of cerebral neurons. the discharge results in almost immediate disturbance of sensation, loss of consciousness or convulsive movements or some combination of the above characteristic (1,2). Seizures are the clinical manifestation of an abnormal with excessive activity of cortical areas that reflect spasmodic events generated by a change in the balance of inhibition and excitation at the cortical level. The International League Against Epilepsy (ILAE) adopted task force proposals for the occurrence of at least one epileptic seizure to definition of epilepsy (3).

Four phases of a seizure can be famed. these components include (4–6):

a) Prodromal phase: in this phase, the symptoms start few hours before or even days before the actual seizure and should not be confused with the aura. The symptoms of prodromal phase consist of irritability, headache, insomnia, displeasure, depression or increased activity.

b) Aura: This phase precedes the seizure by seconds or a few minutes. It is the beginning of the seizure occurrence. The symptoms of the aura phase consist of extreme fear, strange epigastric sensations, dreamlike experiences, and/or unpleasant smells. The patient can remember and describe the aura phase very well.

c) Seizure (ictus phase): In almost many seizures there is a loss of consciousness, and the patient may not be able to remember or give any information about the actual seizure.

d) Post-ictal phase: This phase may not be present, or may last for several hours, and sometimes even for days. The symptoms of this phase include deep sleep and waking up with headache, fatigue, irritability, drowsiness, confusion, muscular aches or ataxia.

1.1.1. Epidemiology of Epilepsy

Epilepsy is one of the most prevalent neurological conditions and an important cause of disability and mortality, epilepsy causes a worldwide serious health concern and An estimated 26% of the burden of neurologic disorders and accounting for 1% of the global burden of disease corresponding to lung cancer in men and breast cancer in women. More than 50 million people worldwide suffer from epilepsy. each year, 16 to 134 new-onset epilepsy cases per 100,000 people increase the global burden of epilepsy (7). each year 120 per 100,000 peoples in the USA come with newly recognized seizure at least 8% of the general population will have one seizure. the rate of recurrence of the first unprovoked seizure within 5years ranges between 23% and 80%. about 125,000 new epilepsy cases occurs each year 30 % of these are in people younger than 18 years of age at the time of diagnosis (8).

The incidence, prevalence and mortality of epilepsy fluctuate with age and place contributing to the variability of the burden of the disease (9) the rate of incidence fluctuates wildly depending on age, a bimodal distribution is constantly noted in studies done in the developed world, the incidence is highest during early childhood particularly in the first year of life and with a decline in

adolescence, the rate of incidence is lowest between the ages of 20 and 40, then gradually rises after fifties, with the highest incidence in people older than 80 (10)**.** In older patients the incidence of epilepsy was highest in those who had experienced a stroke , in the first year after a stroke the developing of epilepsy can increase 20-fold (11).

The prevalence of epilepsy varies significantly between the developed and developing countries, in a systematic review and meta-analysis of published reports, the median lifetime prevalence for developed countries was 5.8 per 1000 in comparison to 15.4 per 1000 for rural and 10.3 per 1000 for urban studies in developing countries (9). The epilepsies, as a whole, are slightly more common in males as compared with females a variety of factors have been anticipated to interpretate for this difference, including lifestyle and environmental considerations. however, the epilepsies are a varied group of conditions that differ in symptomology, etiology, and prevalence across sex (12).

Like other diseases, disability burden was calculated for epilepsy based on the availability of treatments and the presence of seizures (9). A total of 28,300,000 epilepsy cases are expected to live with epilepsy in the entire world in reference to published reports and modeling. the disease ranks fourth after tension-type headache (1,432,500,000), migraine (1,014,000), and Alzheimer disease (43,000,000) (9,13) . The seizures in almost two-thirds of patients with epilepsy can be successfully controlled with currently available antiepileptic drugs (AEDs), leaving one-third with uncontrolled epilepsy (7), while up to 24% of epileptic patients with drug-resistant epilepsy can achieve remissions for more than 1 year (14,15).

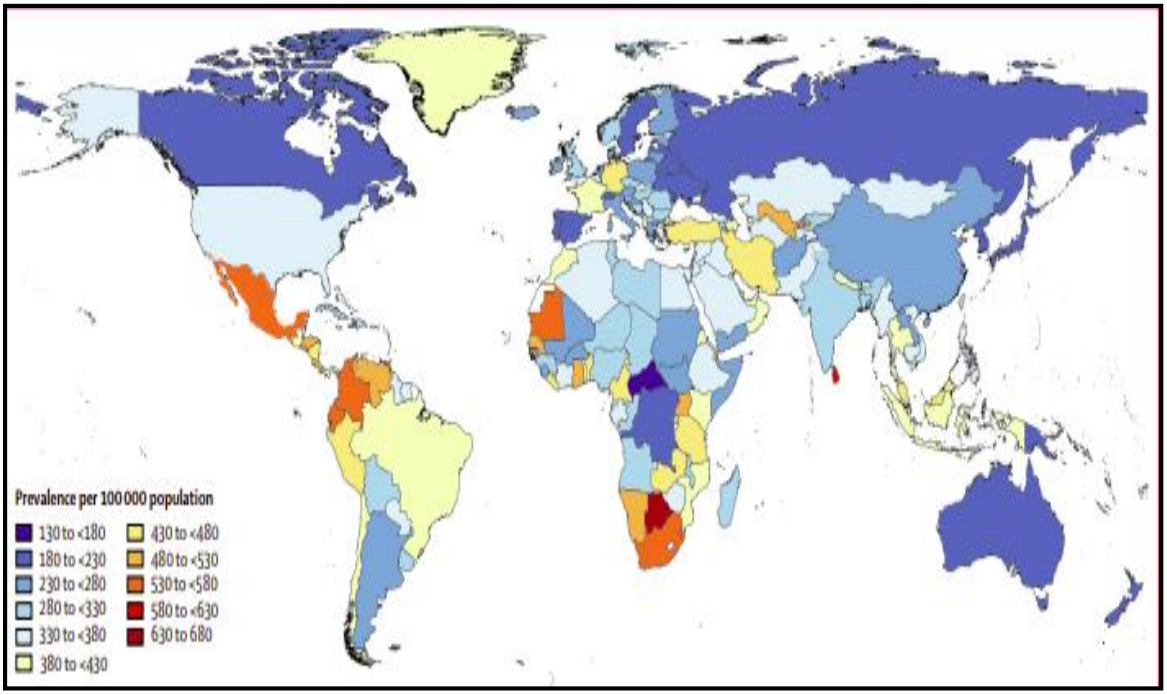


Figure (1-1): Global age-standardized prevalence per 100 000 of idiopathic epilepsy for both sexes, 2016 (16)

1.1.2. Pathophysiology of Epilepsy

Such factors enhancing glutamate signaling which cause increase neuronal excitability or decrease inhibitory control through different mechanisms, and according to the etiology of seizures. Neuronal alterations by sodium (Na+), potassium (K+), calcium (Ca2+), and chloride (Cl−) channels, being the key determinants of membrane potential and neuronal excitability, have all been associated with epilepsy when mutated, to the point that the term “channelepsy” has been proposed (17). A seizure can be hypothesized as occurring when there is distortion of the normal balance between excitation and inhibition in the brain by many mechanisms may include neuronal, biochemical, and network alterations (18). This Excitation/Inhibition imbalance can result

from a modification at many levels of brain function, from genes and subcellular signaling cascades to widespread neuronal circuits. According to the etiologies that adjust Excitation/Inhibition balance can divided the epilepsies into three main categories (18)

1-Seizures can be genetic (idiopathic)leading to epilepsy can occur anywhere from the circuit level (e.g., abnormal synaptic connectivity in cortical dysplasia) to the receptor level (e.g., abnormal g-aminobutyric acid [GABA] receptor subunits in Angelman syndrome) to abnormal ionic channel function (e.g., potassium channel mutations in benign familial neonatal epilepsy [BFNE]) (18,19) Even in the normal developing brain, excitatory synaptic function develops before inhibitory synaptic function, favoring enhanced excitation and seizure generation. In addition, early in life, the neurotransmitter GABA causes excitation rather than inhibition (20). ·

2- Seizures can be acquired due to other causes including CNS infections, hypoxic-ischemic encephalopathy, congenital CNS abnormalities, fever, metabolic and cerebrovascular disorders, trauma, and brain tumors medications, and abused substances (9), Correspondingly, acquired cerebral insults can alter circuit function (e.g., structural alteration of hippocampal circuitry following prolonged febrile seizures or head trauma) (19)

Cerebrovascular diseases contributed to 50–70% of cases and are the single most common cause of adult epilepsy. Recent study from the USA has been reported that the risk for epilepsy occurrence is highest in people with cerebrovascular disease aged 75–79, with African Americans at mostly high risk (21). The exact pathophysiology of stroke-related epilepsy is not established, but intracerebral hemorrhage, hemorrhagic transformation due to ischemic

stroke, severity of stroke, cortical involvement and venous sinus thrombosis are all increases the risk of seizures (22,23).

3-Cryptogenic epilepsy as an epilepsy of presumed symptomatic nature in which the cause has not been identified. The number of such cases is diminishing, but currently this is still an important category (24).

1.1.3. Clinical Presentation of Epilepsy

Detailed classification of quantitative and qualitative clinical events during an epileptic seizure is complicated because of the wide range of possible manifestations, brain maturity, the site of onset in the brain, mechanisms of propagation, sleep–wake cycle, complicating disease processes, drugs, and a range of other factors all influence seizure presentation. Sensation, motor, and autonomic function, consciousness, emotions, memory, cognition, and conduct are all affected by seizures. not all seizures have an impact on all of these factors, but they all have an impact on at least one (3).

1.1.4. Risk Factors of Epilepsy

Risk factors for developing epilepsy can be summarized by problems or injuries in the brain: cerebral blood vessels abnormality ,serious cerebral injury , cerebral hypoxia, hemorrhagic stroke, cerebral infections like meningitis, encephalitis, brain tumor, encephalitis, congenital cerebral diseases, pre-term newborn having seizures in the first month of life , having seizures within days after a head injury (i.e. early posttraumatic seizures), febrile seizures that last longer than usual, long episodes of seizures or repeated seizures (i.e., status epilepticus), family history of epilepsy or febrile seizures, using illegal drugs, like cocaine .other health conditions that make epilepsy more likely: Autism

spectrum disorders, cerebral palsy intellectual and developmental disabilities, Ischemic stroke, Alzheimer's disease (late in the illness) (25).

1.1.5. Classification of Epilepsy

The International League against Epilepsy has recently adjusted its classification of seizures and epilepsies, epilepsy can be classified according to the type of seizure (26), based on whether consciousness is preserved or impaired and whether there is motor involvement. Focal seizures networks originate within subcortical structures that are restricted to one hemisphere with preserved awareness are known as "simple partial" seizures and the focal seizures with impaired awareness are known as "complex partial" seizures. Generalized seizures arising from networks at some point engaged rapidly and distributed bilaterally hemispheric onset seizures that lead to bilateral motor involvement have a stiffening (tonic) phase, followed by a muscle jerking (clonic) phase are known as tonic-clonic seizures (27) as clarified in details figure (1-2).

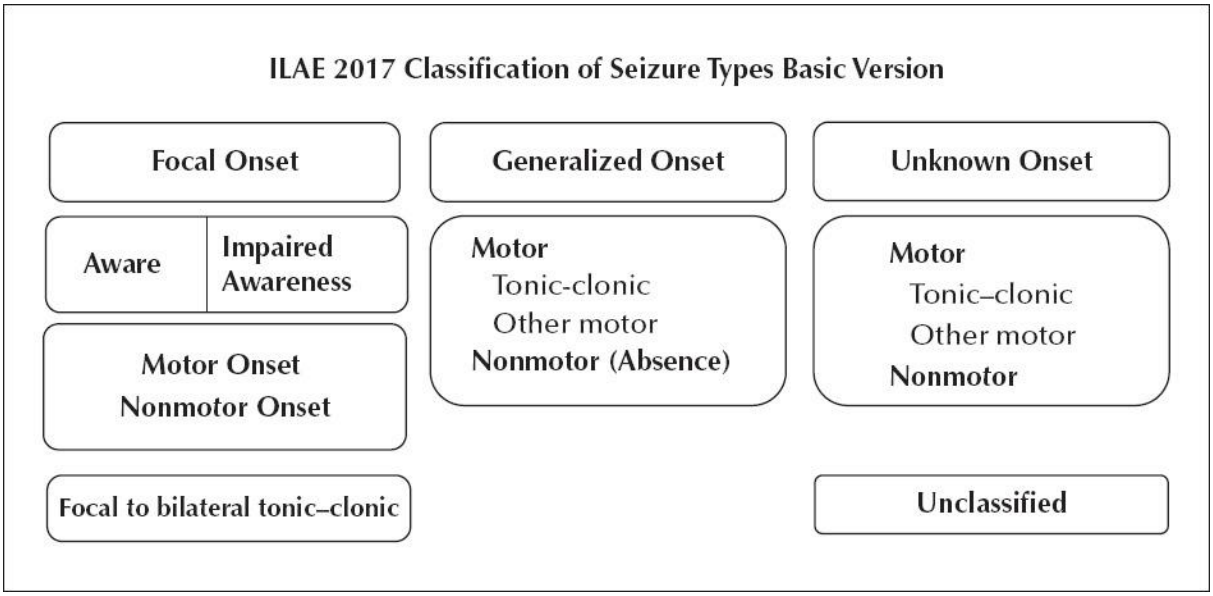


Figure (1-2) ILAE 2017 Classification of Seizure Types (26)

1.1.6. Diagnosis of Epilepsy

Epilepsy diagnosis is clinical based, with the electroencephalography (EEG) findings supporting the diagnosis if positive, but not excluding it if negative. Up to 20% of patients with the clinical diagnosis of epilepsy would have a normal EEG, while only 2% of the patients could have characteristic spike-and-wave EEG abnormalities. Mirsattari et al., recently showed that, even when the routine EEG showed no abnormalities and with normal head computed tomography (CT) scan, the comprehensive assessment including brain magnetic resonance imaging (MRI) and video-EEG telemetry could be of a major importance (28). The EEG is mostly important for identifying specific seizure types. CT scan may help in assessing newly diagnosed patients, but MRI is preferred to locate brain lesions or anatomic defects that are missed by conventional radiographs or CT scans (29).

Besides EEG, neuroimaging studies play an essential role in evaluation of seizures by determining whether it is caused by structural or functional factors and to identify and classify the seizure type and etiology, Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET), single photon emission computed tomography (SPECT), Magneto Encephalogram (MEG), and neuropsychiatric testing are all using in diagnosis and evaluation of seizures (27).

1.1.7. Comorbidities of Epilepsy

Crudely about 50% of adults with active epilepsy have at least one comorbid medical disorder (5,7). Numerous large population-based studies had been reported several conditions that are up to eight times more prevalent in people with epilepsy relative to the general population, obligation of the relevance of these comorbidities is increasing because they affect epilepsy prognosis and patients' quality of life (30,31). To assess the prevalence of nonpsychiatric comorbidities, CDC(center of disease control and prevention) analyzed data from the 2010 National Health Interview Survey (NHIS) adults with epilepsy had a higher prevalence of respiratory, cardiovascular, some inflammatory, and other disorders (e.g., migraine, headache, and many other types of pain) than adults without epilepsy (32). The standardized mortality ratio is significantly increased in the first 15years in patients with coexisting cerebrovascular disease, and after 20 years in those with ischemic heart disease (33).

The major neurobiological comorbidities that have been reviewed with epilepsy including cognitive impairment (34,35) and autism (36). An interesting observation regarding the lower IQ score with a longer epilepsy duration is that the relationship applies to the education (37,38). Therefore, this finding would suggest that interference of early onset epilepsy is associated with developmental cognitive problems and delaying achievement of knowledge and skills in terms of a loss of previously acquired functions (37).

Epileptic patients show an increased prevalence of mental health impairments that have been conversationally termed "psychiatric comorbidities"(39) Mood and behavioral problems are a very common

comorbidity (40) psychiatric comorbidities and migraine are for example associated with poor seizure outcome, whereas depression and anxiety have been linked with reduced quality of life (41,42). So uncontrolled seizures, recurrent seizures, chronic seizures, and even epileptic encephalopathies were considered closely related to progressive cognitive decline, mental problems, and behavioral deficiencies (43,44), but these topics are still controversial and need more evidence.

1.1.8. Management of Epilepsy

1.1.8.1. Non pharmacological Management

The two non-pharmacological surgical therapy options for epilepsy are neuroblation and neuromodulation: The following are common techniques classified as: (45–50)

a) Radiofrequency (RF) thermal coagulation: This method is conducted with the aid of an RF generator attached to the electrode contacts.

b) Magnetic resonance-guided focused ultrasound surgery: is an accurate way of delivering high doses of transcranial ultrasound energy to a distinct intracranial focal point made up of 1024 ultrasound elements.

c) Laser ablation: This can also be accomplished using MRI-guided laser interstitial thermal treatment. The commercially available Visualize Thermal Therapy Device combines a 15W 980 nm diode laser and cooled laser application system with an image processing workstation. The applicator is inserted to reach the target, and laser therapy is performed in the MR scanner, with MR thermal imaging used to visualize the thermal ablation.

d) Stereotactic radiosurgery is a well-established procedure that uses targeted ionizing radiation to target deep-seated lesions and sparing surrounding tissue. ionizing radiation disrupts chemical bonds, resulting in the creation of free radicals.

e) Neuromodulation Functional neurosurgery is the surgical modulation of brain behavior through stimulation or removal of the set of neurons This includes the following:

I. vagal nerve stimulation.

II. Deep brain stimulation.

f) Diet therapy: A low-carbohydrate, high-fat diet is beneficial in the treatment of focal and generalized epilepsies, with 22-55% of patients reporting at least a 50% reduction in seizures on the standard ketogenic diet, after three months of proper nutrition therapy, approximately 50% and 70% of patients show a reduction in seizure frequency of >90% and >50%, respectively

Other types of nutritional therapy are available, such as the medium-chain triglyceride diet and the low glycemic index diet. Many patients report better cognition and mood, as well as seizure control, while on nutritional therapy.

1.1.8.2 Pharmacological Management

There are over 25 different AEDs used clinically to treat patients with epileptic siezure, has been approved by the U.S. Food and Drug Administration (FDA) (51). However, only about half of the patients with a newly diagnosed epilepsy are effectively treated with the first prescribed AED, and about one third of patients with epilepsy suffer from intractable seizures (52) In a population-based study for assessing antiepileptic drug resistance which conducted in Western Europe, the epilepsy in 22.5% of all patients was found to be drug-resistant (7). The mechanism of drug resistance has not been fully explained, but genetic factors have been recognized as the most important cause of drug efficacy differences in the treatment of epileptic patients (53). Drug-metabolizing enzymes, transporter, and receptor variations may be due to genetic polymorphisms that affect the clinical outcomes of epilepsy in different antiepileptic drugs (56), and these clinical consequences of gene polymorphisms associated with antiepileptic drugs have been reported (55,56).

There are currently more than 20 antiepileptic medicines available to treat epilepsy. antiepileptic drug selection is generally based on evidence of efficacy and effectiveness for the patient's seizure type, but additional patient-specific factors such as age, sex, comorbidities, childbearing potential, and concurrent drugs must also be properly considered (57). Mechanisms of action of selected AEDs were shown in table (1.1) and figure (1-3) (58).

Table (1.1) Mechanisms of action of selected AEDs used for the treatment of epilepsy

Mechanism	Antiepileptic drugs
Block repetitive activation of sodium channels	Phenytoin, carbamazepine, oxcarbazepine, lamotrigine, topiramate
Enhance slow inactivation of sodium channels	Lacosamide, rufinamide
Enhance activity of g-aminobutyric acid (GABAA) receptors	Phenobarbital, benzodiazepines, clobazam
Block N-methyl-Daspartate (NMDA) receptors.	Felbamate
Block a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors	Perampanel, topiramate
Block T-calcium channels	Ethosuximide, valproate
Block N- and L-calcium channels	valproate, Lamotrigine, topiramate, zonisamide
Modulate H-currents	Gabapentin, lamotrigine
Block unique binding sites	Gabapentin, levetiracetam
Inhibit carbonic anhydrase	Topiramate, zonisamide
Open potassium channels (KCNQ [Kv7])	Retigabine
Inhibit GABA transaminase	Vigabatrin
Inhibit GABA reuptake	Tiagabine

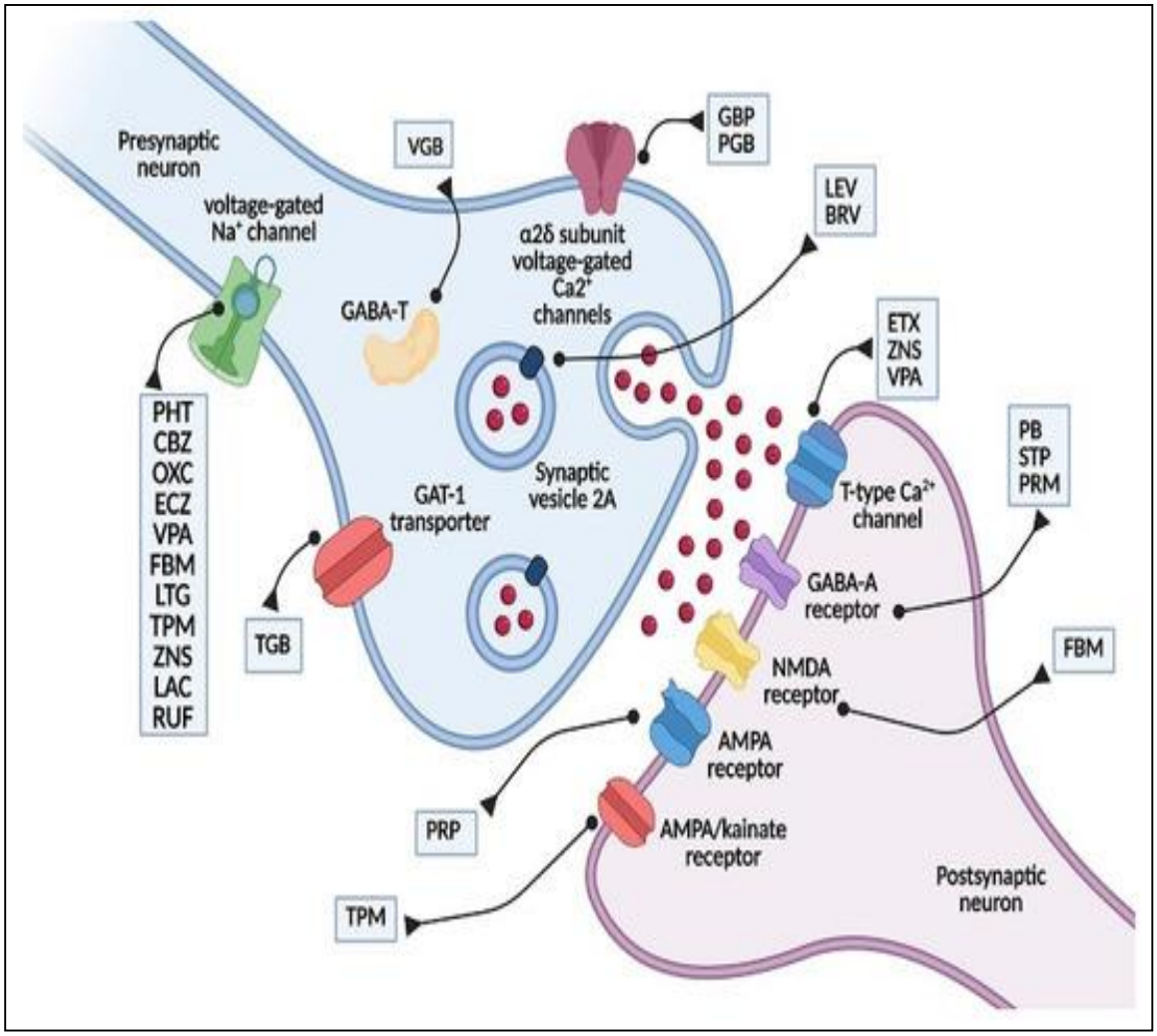


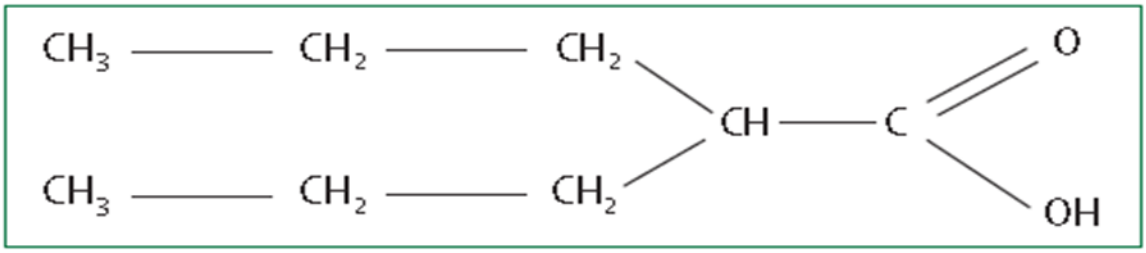
Figure (1-3) Mechanism of action of antiepileptic drugs

1.2. Valproic Acid

Valproic acid (VPA) (2-n-propylpentanoic acid) is fatty acid having an anticonvulsant property is one of the most widely used AEDs in different countries of the world (59), and it can be prescribed as either monotherapy or as part of polytherapy regimens comprising several AEDs ,VPA used for the treatment of numerous types of epilepsy in pediatrics and adults (60,61). Even though VPA is an effective drug and usually well

tolerated, it shows high interindividual variability in dose and steady-state serum concentration, which in turn affects its therapeutic effects. Therefore, the VPA serum concentration needs to be monitored during the treatment (62). Many studies have illustrated that lower plasma concentrations of antiepileptic drugs could be the main factor of pharmaco-resistant epilepsy with VPA treatment (63)

The first line of pharmacological management of epilepsy is monotherapy because of its effectiveness, well tolerance and associated with low costs, higher quality of life besides the better patient compliance (64,65). Unblinded study was designed by The Standard and New Antiepileptic Drugs (SANAD) trial, for long-term (up to 6 years) which stated that valproic acid (VPA) was identified as a first-line treatment (64).



Figure(1-4) Chemical structure of valproic acid (66)

1.2.1. Clinical Use of Valproic Acid

Valproic acid is FDA approved antiepileptic drug, Besides its antiepileptic clinical use and beneficial effects in clinical depression absence seizures, tonic-clonic seizures, complex partial seizures, juvenile myoclonic epilepsy, seizures associated with Lennox-Gastaut syndrome (67,68), VPA can be used in psychiatric care to treat mania in patients diagnosed with bipolar

either alone or in combination with other antipsychotic agents(69), mood stabilizing drugs (70) migraine headaches (71), and schizophrenia (72,73).

1.2.2. Valproic Acid Pharmacokinetics

Valproic acid is rapidly absorbed after oral administration. Administration with food does not affect total absorption. Peak serum concentration occurs approximately 0.5 to 2.5 hours after an oral dose. Steady state is reached in 2 to 4 days (74). Valproic acid is bound very strong (90%) to human plasma proteins (mainly albumin) (74). Due to the saturable plasma protein binding, the relationship between dose and total valproate concentration is nonlinear. However, the kinetics of unbound drugs (not bound to proteins) is linear. Valproate is almost totally metabolized by the liver and excreted in urine, The serum half-life is between 6 and 16 hours (74).

Therapeutic drug monitoring **(**TDM) is required to assure VPA's efficacy and safety. when assessed clinically the therapeutic range for total VPA serum levels is 50–100 mcg/mL (75). VPA is highly bound to albumin, and when serum concentration increased, the binding capacity becomes saturated, resulting in increasing levels of free (unbound) VPA. The drug's pharmacologic effects, as well as its toxicity, are related to free VPA. The unbound fraction of VPA is approximately 10% of the total serum concentration at a normal albumin concentration and modest total serum concentration. When total serum concentration is greater than 100 mcg/mL, the unbound fraction may exceed 15% (76). The presence of endogenous substances that compete with VPA for albumin (e.g., bilirubin, fatty acids, and urea nitrogen) and concomitant medications (e.g., aspirin, nonsteroidal anti-inflammatory drugs, phenytoin,

warfarin, propofol, clevidipine, and lipid infusions) may increase the unbound fraction of VPA and potentially increasing toxicity (77).

1.2.3. Valproic Acid Pharmacodynamics

Epilepsy is an ion channel disease concerning multiple ion channels, such as chloride, calcium, potassium, and sodium. the pharmacological basis of the antiepileptic action of VPA has been related to the regulation of the glutamate excitatory neurotransmission and gamma-aminobutyric acid (GABA) inhibitory neurotransmission (78,79), VPA attenuates N-Methyl D-Aspartate-mediated excitation and blocks sodium Na+ channels, calcium Ca+ channels, and voltage-gated potassium K+ channels (66,75) . VPA eliminates the high-frequency repetitive electrical activation of central nervous cells and delays the recovery of inactivated sodium ion channels, persistently reducing the electrical conduction of sodium ions (68). in addition, long-term VPA treatment can upregulate the expression of sodium channels on cell surfaces. Moreover, Recent studies showed that VPA is an effective inhibitor of histone deacetylases, the key enzymes for the control of histone acetylation state and hence for the epigenetic regulation of gene expression (80,81) principally

through histone deacetylases inhibition, VPA induces apoptosis of microglia cells and activates BDNF promoter. Furthermore, VPA induces neuronal differentiation but suppresses astrocytic and oligodendrocytic differentiation of neural stem cells and promotes neurite outgrowth (80,82).

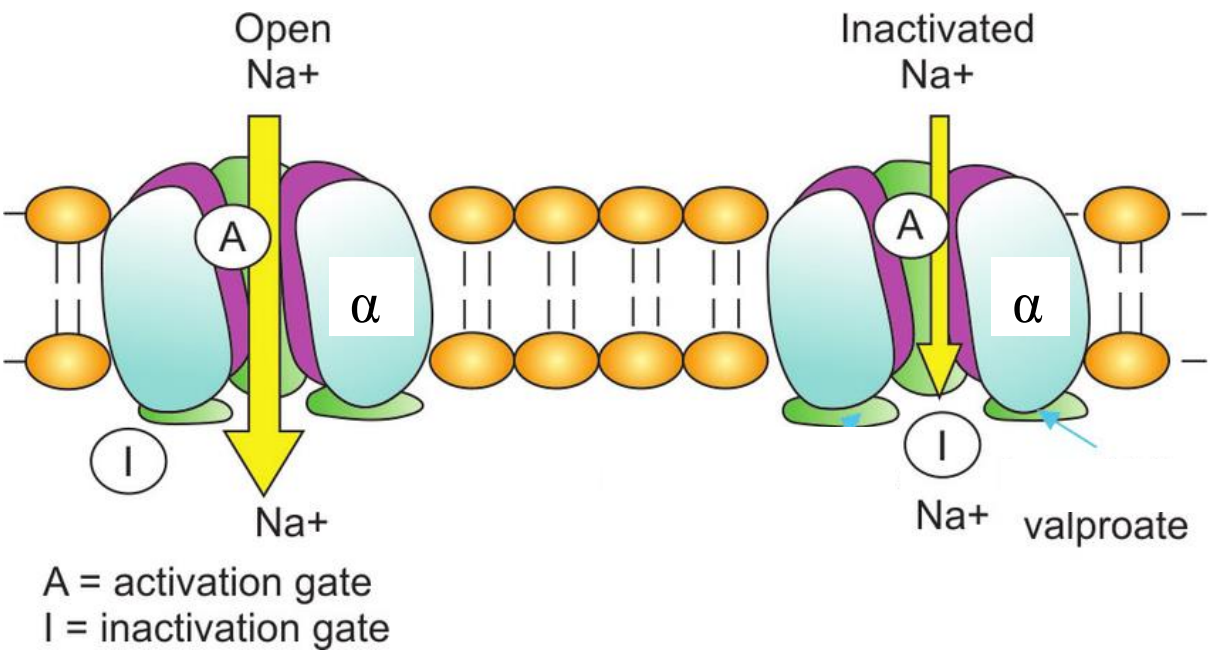


Fig. (1-5) Mechanism of action of valproic acid on Sodium voltage- gated channel alpha subunit 1 (SCN1A) (83)

1.2.4. Valproic acid Adverse Effects

Common side effects of VPA include tremor, sedation, fatigue, gastrointestinal symptoms, and weight gain, these side effects are generally dose or serum concentration dependent and can be minimized by dosage modification (66,84). VPA was concerned about higher rates of teratogenicity and delayed cognitive development in children in utero, lamotrigine (LTG) has been recommended as an alternative to VPA (58). VPA adverse reactions can be categorized as neurological, endocrine, metabolic, and hematological ones (66,85). Hepatotoxicity, teratogenicity, and pancreatitis are all life-threatening adverse drug reactions (ADR) associated with this therapy. hepatotoxicity caused by VPA is linked to changes in VPA metabolism and can be fatal in children, Valproate has been linked to liver damage due to mediated synthesis of

the hepatotoxic metabolite 2-propyl-4-pentenoic acid (4-ene-VPA) and 2-propyl-2,4-pentadienoic acid (2,4-dieneVPA) (86).

1.2.5. Drug Interactions

Some medicines that may interact with this drug include: certain antidepressants (e.g., amitriptyline, nortriptyline, phenelzine), certain antibiotics (carbapenems such as doripenem, imipenem), irinotecan, mefloquine, orlistat, other medications for seizure (e.g., ethosuximide, lamotrigine, phenytoin, rufinamide, topiramate), rifampin, vorinostat, warfarin, zidovudine (87).

1.2.6. Metabolism of Valproic Acid

The metabolism of valproic acid occurs primarily in the liver via three pathways glucuronidation (50%), β-oxidation (40%) and cytochrome P450 (CYP)-mediated oxidation (10%) (88). glucuronidation is a critical step in the metabolism of a variety medicines as well as a variety of endogenous substances like bile acids and steroid hormones. UDP-glucuronosyltransferases(UGTs) are a family of drug metabolizing enzymes contributing to hepatic drug metabolism, UGTs involve four families have been identified in humans involve UGT1, UGT2, UGT3 and UGT8 each one involves many subfamilies which distributed in liver and other extrahepatic organs (89) that are encoded by a family of 19 protein-coding genes is located on chromosome 2q37 and 4q13. Valproate glucuronide is the principal metabolite of VPA in urine (30-50%) and is not considered to be toxic for cells , UGTs enzymes in responsible for of this conjugation reaction. On the contrary, some of the products of VPA metabolism produced by mitochondrial and non-mitochondrial pathways are known to be hepatotoxic (90).VPA is a fatty acid that can be metabolized in the mitochondria

via endogenous mechanisms. Some of the VPA mitochondrial metabolites produced by this route are hepatotoxic. VPA bioactivation include the entry of 4-ene-VPA into the mitochondria, the synthesis of a 4-ene-VPA-CoA ester then -oxidation to produce the reactive 2,4-diene-VPA-CoA ester (91) . this cytotoxic metabolite (2,4-diene-VPA-S-CoA) is then coupled to glutathione to create thiol conjugates. 4-ene-VPA-derived chemically reactive metabolites can deplete mitochondrial glutathione pools and form conjugates with CoA .in turn inhibiting enzymes in the β-oxidation pathway. the detection of N-acetylcysteine conjugates of (E)-2,4-diene-VPA in human urine indicated that the reactive thiol conjugates of VPA are largely produced by (E)-2,4-diene VPA biotransformation in humans (84).

The synthesis of the metabolite 4-ene-VPA by CYP2C9, CYP2A6, and to a lesser extent by CYP2B6 is the most important CYP-mediated branch of the VPA pathway. Furthermore, these metabolizing enzymes are involved in the conversion of VPA to inactive 4-OH-VPA and 5-OH-VPA (92). CYP2A6 is also involved in the production of 3-OH-VPA. The complex metabolism of valproic acid leads to much of its associated toxicity, moreover, a growing number of genes have been confirmed affecting its metabolism, efficacy and safety, which partly illustrate interindividual variability among patients taking VPA some genetic variants also have been observed to have a close relationship with serious side effects, including hepatotoxicity and teratogenicity (93).

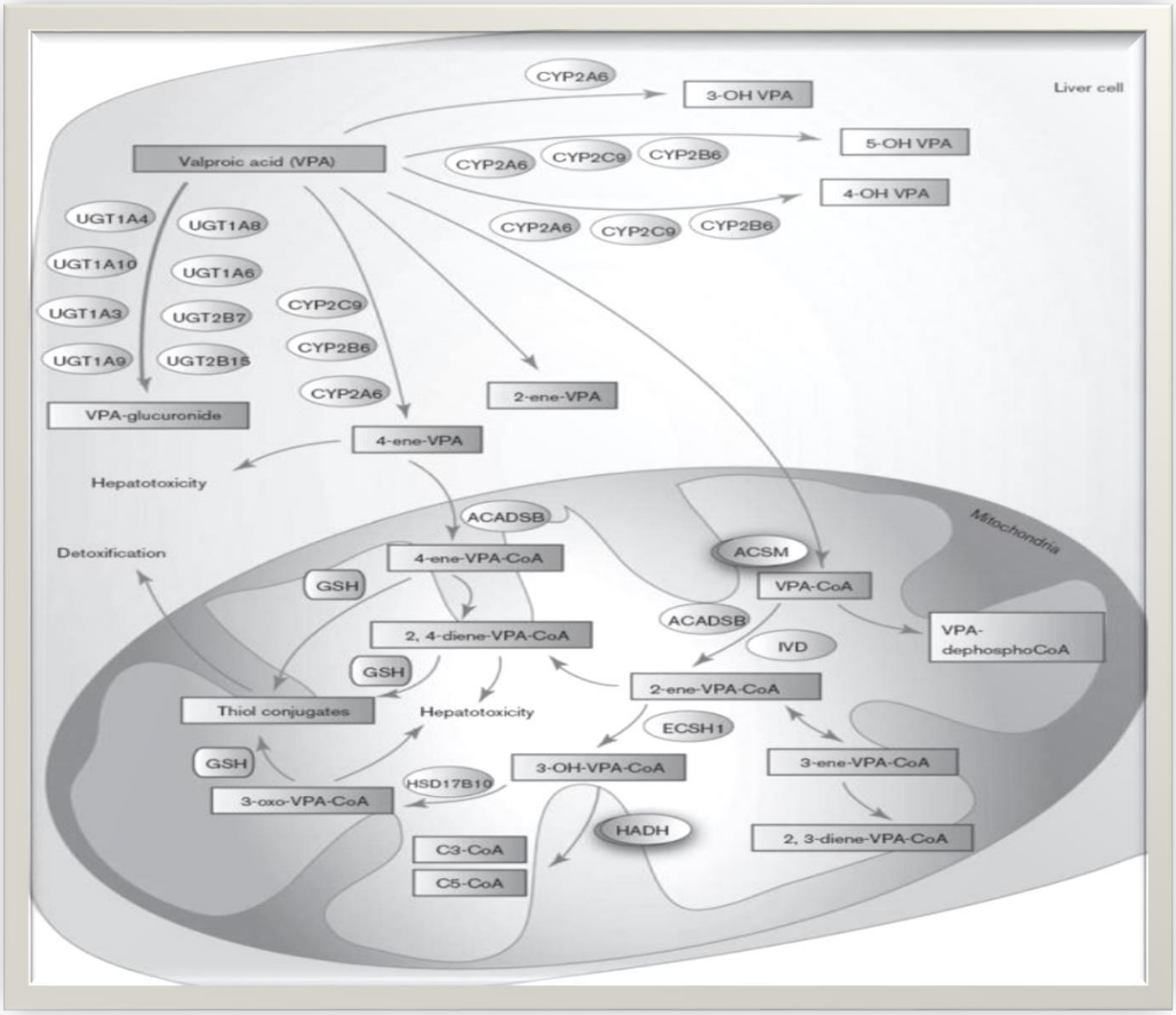


Fig. (1-6): Metabolism of Valproic acid (88)

1.3.Single Nucleotide Polymorphism

SNPs are the most prevalent type of human genetic variation and they are variances in DNA sequence between individuals or communities, that is alterations of a single base occurring at a 1% frequency within a population. Single nucleotide polymorphisms are either passed down from parents to

children as DNA sequences which is known as genetic mutation, which is most often related to diseases or they are created by external sources such as radiation or viruses which is known as mutation (94). SNPs can be synonymous or nonsynonymous. These two types of SNPs occurred in gene coding regions (exon), single nucleotide polymorphism can also occur noncoding (intron) (95). Genetic polymorphisms play an important role in human susceptibility to diseases, such as idiopathic epilepsy (96). as well as interindividual variation in drug response and drug adverse effects, such as pharmacoresistance to valproic acid

1.3.1.Genetic Polymorphism of Valproic Acid Site of Action SCN1A (sodium channel subunit 1-α)

The development of the pharmacogenomics in valproic acid was helpful to identify a large amount of applicant genes, such as receptor gene mutations, regulating signaling pathway-related transporter, enzyme, affecting the pharmacokinetic, pharmacodynamic and toxic reaction of VPA (88).

Voltage-gated sodium (Nav) channels are dynamic for the initiation and propagation of neuronal action potentials (96) and consider a major targets for many first-line AEDs, such as carbamazepine (CBZ), oxcarbazepine (OXC), phenytoin (PHT), lamotrigine (LTG), and valproic acid (VPA) (96,97). Nav channels are composed of both α and β subunits, α subunit is the functional subunit. VPA and other sodium channel blockers bind to the α subunit to exert their therapeutic effects (98). The defects of the sodium channel subunit slow down its inactivation and prolong the time of depolarization of cell membranes,

leading to the generation and propagation of seizures (60,99). The α -subunit is divided into four homologous sections, each with six transmembrane segments (S1–S6). S4 is the voltage zone among them. The gate zone is made up of S5, S6, and the ring that connects them. the SCN1A protein coding area are expressed throughout central nervous system and play a significant role in regulating neuron excitability, particularly any alterations in the gate zone can cause changes in the sodium channel's permeability and electro-conductivity resulting in increased cell excitability and neuronal discharge with small stimulations (100). Gene SCN1A, encoding the α subunit of the Nav1.1 channel mutated most frequently and is associated with genetic epilepsy with a wide range of severities (60,101). Similarly, the SCN2A gene, which codes for the α subunit of the Nav1.2 channel, is closely associated with many types of epilepsy (96).

Genetic variations in SCN1A may be the major determinants of individual phenotypic differences in response to AEDs, which has aroused a great interest among researchers (96,102,103) . the sodium channel subunit 1 is encoded by SCN1A gene which is found on chromosome 2q24 and has 26 exons, rs2298771 (A>G) is the SNP of SCN1A gene and the changing is from Adenine (wild) allele to Guanine (mutant) allele is located in exon 16 (104)

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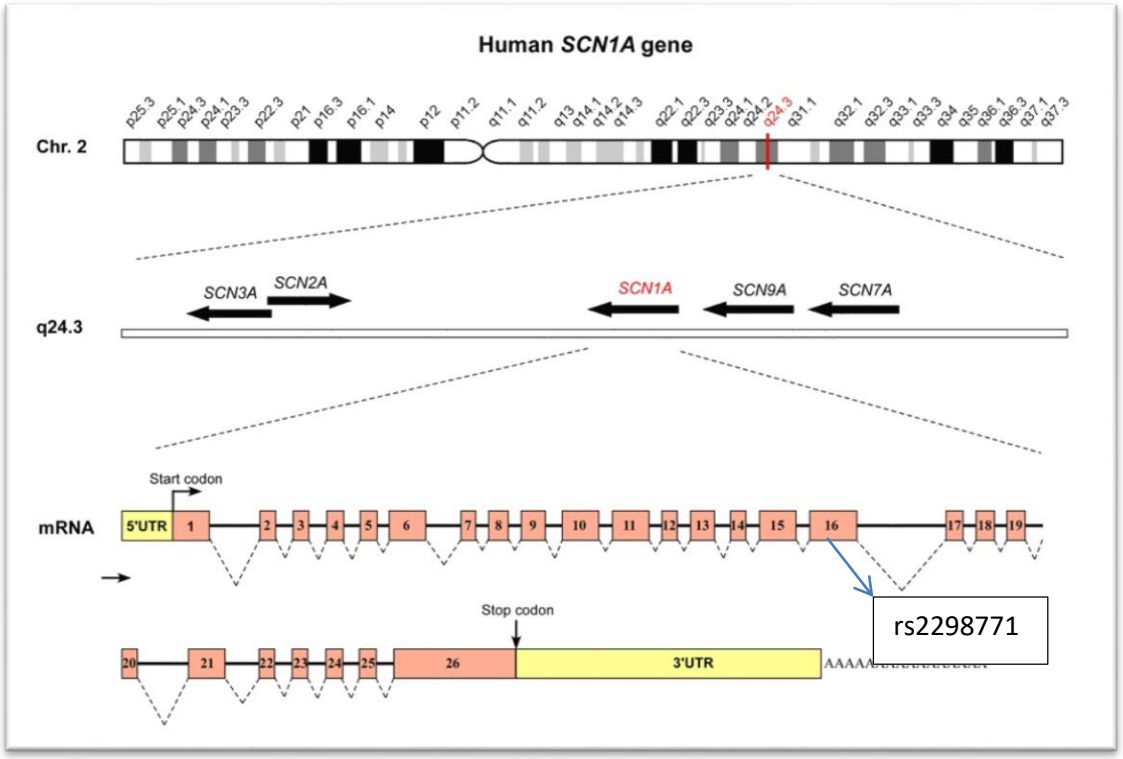


Fig. (1-7) SCN1A gene SCN1A, encoding $Na_v1.1$, is located at position (2q24.3).

1.3.2. Genetic Polymorphism of Valproic Acid Metabolizing Enzyme (UGT2B7)

Valproic acid undergo glucuronidation by UGTs enzymes directly to produce VPA-glucuronide, (106). UGT2B7 has a broad substrate specificity that is expressed in the liver, steroid target tissues and a variety of organs and is an important member of the UGT2B subfamily because it conjugates and metabolism a large variety of compounds including estrogens, fatty acids, bile acids and steroids. androgens, morphine, retinoic acid (107,108) the UGT2B7 enzyme is encoded by UGT2B7 gene which is highly polymorphic with nonsynonymous, synonymous, intronic, and promoter single nucleotide

polymorphisms (SNPs), some of which have been reported to affect UGT2B7 expression and glucuronidation activity (109). UGT2B7 is a gene with a total length of just 16 kb on chromosome 4q13, it consists of six exons. (rs7439366) (C>T) c.802 is the SNP of UGT2B7 gene and the changing is from cytosine (wild) allele to Thiamin (mutant) allele is located in exon 2 Among them, cause substitution of tyrosine to histidine at amino acid 268 in exon 2 resulting to UGT2B7 isozyme as in the figure (1-5) (110). More recent studies have shown that the polymorphisms of UGT2B7 c.802 C>T ,c.161C>T affect the adjusted concentration (AC) of VPA which may have a significant impact on clinical use of VPA UGT2B7 (111,112). There is difference in published reports on the effect of UGT2B7 c.802 C/T polymorphisms on the biological activity (113,114). other SNP such as 161C< T, 735A< G also have been identified to affect VPA metabolism and unpredictable results have been reported regarding toVPA glucuronidation (115,116).

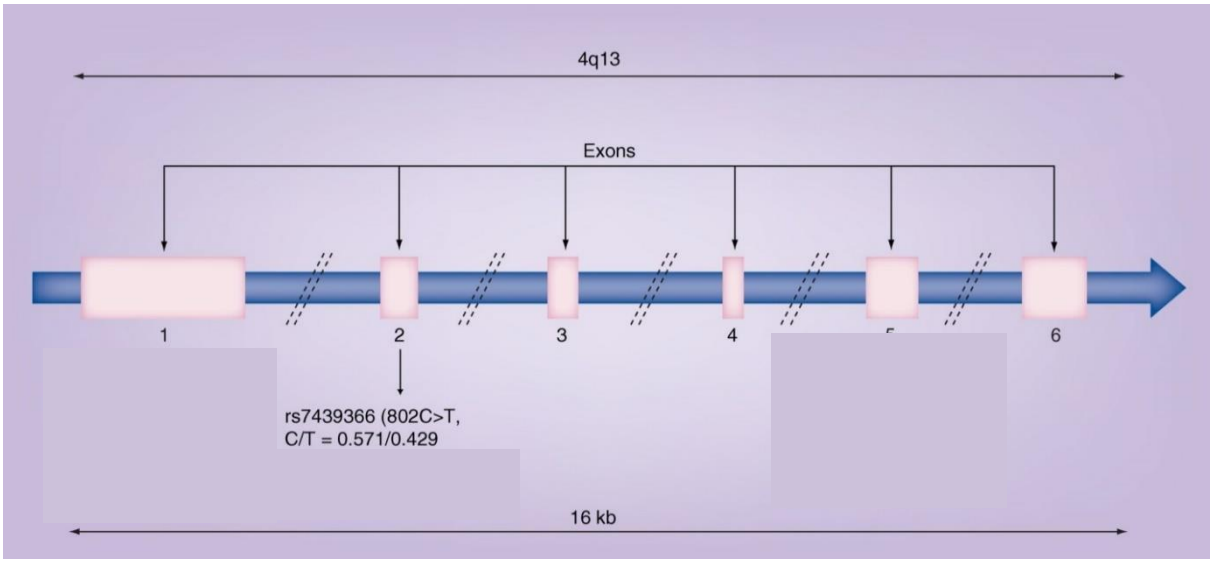


Fig. (1-8) UGT2B7 gene (110)**.**

1.4. Aimes of The Study

1. To investigate the distribution of genetic polymorphism in SCN1A gene c.3184 A>G (rs2298771) (encoding voltage-gated sodium channels) and UGT2B7 gene c.802 C>T (rs7439366) (encoding UGT2B7 metabolizing enzyme) in epileptic patients of Karbala province .

2 .To study the effect of genetic polymorphism of SCN1A gene c.3184 A>G (rs2298771) and UGT2B7 gene c.802 C>T (rs7439366) on valproic acid response and the effect of genetic polymorphism of UGT2B7 c.802 C>T (rs7439366) in development of hepatotoxicity as a drug adverse effect among epileptic patients at Karbala province .

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Subjects (Patients)

This study was conducted on one hundred and thirteen (113) patients diagnosed as epileptic patients by a senior neurologist based on the International League Against Epilepsy's criteria (ILAE) (3), medical history and clinical manifestations many of them were newly diagnosed and many other had previously AED failed treatment. the complete written informed permission taken from each patient after explaining the nature of the study and asked to complete the specially crafted questionnaire.

Only one hundred and three patients (103) returned after three months of treatment to follow up and only ninety patients (90) returned after six months of treatment to follow up due to a coronavirus pandemic and loss of follow up during treatment period so they were excluded from the study.

2.1.1 Study Design

This study is a prospective study carried out at Imam AL-Hussein Medical City / Karbala –Iraq in Neurology consultant Center, out clinic patients ,out laboratory and laboratories of College of Pharmacy / University of Karbala. during the period from October 2020 to November 2021

2.1.2. Patients Criteria

2.1.2.A Inclusion Criteria

Patients involved in the study should have the followings:

1. Patients who had epilepsy generalized or focal seizures.
2. Age range from 2 to 60 years.
3. Initially prescribed VPA monotherapy at a therapeutic dose and continuing treatment for 6 months duration.

2.1.2.B Exclusion Criteria

1. Patients who had severe adverse drug reactions and who had severe renal, hepatic, or cardiac dysfunction.
2. Patients who received other drugs that affect valproic acid response and/or may impact VPA metabolism (drugs affected on UGT2B7 activity inducers or inhibitors) were excluded from the study.
3. Drug abuse or history of alcohol.
4. Pregnant women and breast feeding female patients.
5. Incomplete seizure frequency record or failure to follow-up during the study period.

2.1.3. Clinical Data Collection

All enrolled patients were diagnosed as epileptic patients and classified by a senior neurology specialist based on the International League Against Epilepsy's criteria (ILAE) (3,117), electroencephalograms (EEG) , magnetic resonance imaging (MRI), and computed tomography (CT) scan, and family history of epilepsy as well as clinical manifestations such semiology, available home recordings and eyewitnesses. Demoghraphic parameters (questioner) were taken from all patients at the study beginning which were: age, weight, gender, age at onset of seizure, family history of epilepsy, medical and neurological history, frequency and duration of seizures, and previous epilepsy treatment if found. Epilepsy diagnosed and classified.

Clinical data were collected monthly at each visit from each patient's or parent or guardian) kept a record of the date, number of seizure attacks on a daily record card, re-evaluation processes were done in third and sixth months after enrolment. 3-month retrospective baseline was used to establish the baseline seizure frequency.

Patients were initially prescribed the extended release ER formulations of valproic acid (Depakine Chrono) tablet and (Depakine) syrup. at initially, the median dose was 10 mg/kg, which may increase to 15 mg/kg at subsequent visits. The physician determined the dose, frequency of dosage, initial and maintenance and any additional increase or decrease dose based of their seizures control. If a patient entered the study on antiepileptic drug other than VPA, it should be tapered off gradually within two weeks after the initiation the study .

The efficacy measures include the percentage change in seizure frequency depending on registered numbers of seizure attacks of any type per month from

baseline through the treatment period, seizure frequency is the most sensitive measure of AED efficacy and should be used whenever possible (118,119). The patients were divided into two groups based on their response over three months conducted to 103 patients and over six months conducted to 90 patients, this group are :

1-VPA-responsive patients had response rate ≥ 50% [reduction in number of seizure attacks more than 50% from baseline]

2-VPA- poor responsive patients had response rate < 50% [reduction in number of seizure attacks less than 50% from baseline].

In third month, VPA-poor responsive patients (who had inadequate seizure control) were increased valproic acid dose based on their prognosis by neuro physician keeping in mind serum levels of valproic acid.

In sixth month, VPA-poor responsive patients (who had inadequate seizure control) were converted to another antiepileptic drug or to a polytherapy regimen at the end of the study.

2.2. Materials

2.2.1. Kits and Chemicals

The kits and specific chemicals with their origin and manufacture are listed in table (2-1)

Table(2.1): chemicals and kits and their manufacturing companies

Kits and Chemicals	Manufacture	Origin
Absolute Ethanol	SDI	Iraq
PCR PreMix Kit	Bioneer	Korea
Agarose powder	Bio Basic	Canada
Nuclease free wate	Bioneer	Korea
Deionized water	Bioneer	Korea
DNA Ladder (100bp)	Bioneer	Korea
Ethidium bromide	Intron	Korea
G-spin DNA extraction kit	Intron	Korea
Primers	Bioneer	Korea
(TBE) Buffer	Bioneer	Korea
Serum VPA concentration kit	Abbott	Germany
Serum AST concentration kit	Abbott	Germany
Serum ALT concentration kit	Abbott	Germany
Serum Total Bilirubin concentration kit	Abbott	Germany

2.2.2 Instruments

The instruments utilized in the genetic and biochemical examination in this study are listed in table (2-2) with their origins and manufactures

Table 2.2 the Instrument and Apparatus and their manufacturing companies

Instrument	Manufacture	Origin
Incubator	Binder	Germany
Centrifuge	Hettich	Germany
Digital camera	Canon	England
Gel Elctrophoresis apparatus	Cleaver	UK
PCR machine – thermocycler	Veriti	United States
Freezer	Concord	Lebanon
Micropipettes	Slammed	UK
Sensitive balance	AND	Taiwan
UV – Trans illuminator	Syngene	England
Vortex mixer	Human Twist	Germany
Water path	LabTEch	Korea
ARCHITECT i1000	Abbott	Germany
ARCHITECT c4000	Abbott	Germany

2.3. Method

2.3.1. Samples Collection

Three blood samples were collected from each patient in the outer laboratory or in patients 's home by a medical representative from laboratory in scheduled time.

a) First sample before the treatment:

venous blood sample (5ml) was taken from each patient (n=113), (2 ml) was kept in an ethylene diamine tetracetate (EDTA) tube stored frozen until used for DNA extraction and subsequent genotyping, and (3ml) was kept in a gel tube to isolate serum by centerfugation of the blood at 3000 rpm for 10 minute used for liver enzymes tests before the treatment.

b) Second sample after three months of treatment:

venous blood sample (3ml) was taken from each patient (n=103) in the morning before the next dose of valproic acid and in the steady state condition kept in gel tubes, the serum was separated for estimating concentration of valproic acid in the serum .

c) Third sample after six months of treatment

venous blood samples (3ml) were taken from each patients (n=90) in the morning before the next dose at steady state kept in gel tubes the serum was separated for estimating serum concentration of valproic acid and for liver enzymes tests.

2.3.2 Biochemical Assay

2.3.2.1. Estimation of Serum Valproic Acid Level

Immunoassay for the vitro quantitative determination of valproic acid in human serum was used in this study, the ARCHITECT iValproic Acid assay in vitro using chemiluminescent microparticle immunoassay (CMIA) technology for the quantitative measurement of valproic acid in human serum or plasma.

Biological principle

1. Sample anti-valproic acid coated paramagnetic microparticles, and valproic acid acridinium-labeled conjugate are combined to create a reaction mixture. the anti-valproic acid coated microparticles bind to valproic acid present in the sample and to the valproic acid acridinium-labeled conjugate.
2. After washing, pre-trigger and trigger solutions are added to the reaction mixture.
3. The resulting chemiluminescent reaction is measured as relative light units (RLUs). an indirect relationship exists between the amount of valproic acid in the sample and the RLUs detected by the ARCHITECT i System optics.

The assay consists of the following reagents:

- microparticles: anti-valproic acid (mouse, monoclonal) coated goat anti-mouse microparticles in TRIS buffer with protein (bovine)

stabilizer minimum concentration : 0.1 % solids and preservative; Proclin 950

- conjugate :valproic acid acridiniumlabeled conjugate in buffer with surfactant minimum concentration 40.0 ng/ml and preservative; Proclin 950.
- pre-Trigger solution: ARCHITECT i PreTrigger Solution containing 1.32% (w/v) hydrogen peroxide.
- Trigger solution: ARCHITECT i Trigger Solution containing 0.35 N sodium hydroxide.
- Wash buffer ; ARCHITECT i Wash Buffer containing phosphate buffered saline solution and preservative antimicrobial agent.

Trough concentration was measured by direct chemiluminescence assay, with a linear range of (2 -150 µg/ml). The VPA concentration at steady-state was adjusted by dosage and body weight of each patient and expressed as AC (adjusted concentration) due to large inter-individual variances, and to eliminate the error from weights and dosages .AC calculated by this equation (62):

AC (µg/mL per mg/kg) = serum VPA concentration (µg/mL)/[VPA daily dose (mg per day)/weight (kg)]

2.3.2.2 Estimation of Serum Liver Enzymes Level

2.3.2.2.A. Estimation of Serum Aspartate Aminotransferase (AST) level

Aspartate aminotransferase enzyme in human serum was measured by the ARCHITECT c 4000 Systems (120)

Biological Principles

AST present in the sample catalyzes the transfer of the amino group from L-aspartate to α-ketoglutarate, forming oxaloacetate and L-glutamate. Oxaloacetate in the presence of NADH and malate dehydrogenase (MDH) is reduced to L-malate. In this reaction, NADH is oxidized to NAD. the reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD. methodology: NADH .The assay consists of the following reagents:

Reactive Ingredients Concentration

R1

- β-NADH 0.16 mg/Ml
- Malate dehydrogenase 0.64 U/mL
- Lactate dehydrogenase 0.64 U/mL
- L-aspartate 232 mmol/L

R2

- α-ketoglutarate 51.3 mmol/L
- L-aspartate 100 mmol/L

2.3.2.2.B. Estimation of Serum Alanine Aminotransferase (ALT) level

The Alanine Aminotransferase (ALT) assay is used for the quantitation of alanine aminotransferase in human serum or plasma was measured by the ARCHITECT c4000 System (121).

The Principles

ALT present in the sample catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate, forming pyruvate and L-glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase (LD) is reduced to L-lactate. In this reaction NADH is oxidized to NAD. the reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD. Methodology: NADH

Reagents

Reactive Ingredients Concentration

R1

- β-NADH 0.16 mg/mL
- Lactate dehydrogenase 2.57 U/mL
- L-alanine 392 mmol/L

R2

- α-Ketoglutaric acid 77 mmol/L
- L-alanine 1,000 mmol/L

2.3.2.2.C. Estimation of Serum Total Bilirubin level

The Total Bilirubin assay is used for the quantitative analysis of total bilirubin in human serum of adults and neonates on the ARCHITECT c 4000 Systems .

The Principles

Traditional methods of measuring bilirubin are based on the reaction of bilirubin with a diazo reagent to form the colored compound azobilirubin. The diazo reaction can be accelerated by the addition of various chemicals. For example, Malloy-Evelyn3 used methanol, Jendrassik-Gróf4 used caffeine and Walters-Gerarde5 used dimethyl sulfoxide (DMSO). modifications of these methods included the addition of surfactants as solubilizing agents. total (conjugated and unconjugated) bilirubin couples with a diazo reagent in the presence of a surfactant to form azobilirubin. the diazo reaction is accelerated by the addition of surfactant as a solubilizing agent. the increase in absorbance at 548 nm due to azobilirubin is directly proportional to the total bilirubin concentration.

Methodology: Diazonium Salt

Reagents

Reactive Ingredients Concentration

R1

- Surfactants 4.53%
- HCl 9.33 g/L

R2

- 2, 4-dichloroaniline 0.81 g/L
- HCI 5.563 g/L
- Sodium nitrite 0.345 g/L
- Surfactant 1.96%

2.3.3 Molecular Analysis

2.3.3.1 Extraction of Genomic DNA

The genomic DNA was obtained from a blood sample by using blood genomic DNA extraction kit (G- spin Total DNA extraction) from Intron Company:

1. solution of 200 µl of the whole blood, 20 µl proteinase K and 5 µl RNase were placed in a 1.5 ml microcenterfuge tube and mixed using a pulse vortex.

2. Into the upper sample tube add 200 µl of Buffer BL and thoroughly mix to ensure efficient lysis solution.

3. set the mixture at room temperature for 2 minutes and allow to cool, the lysate is then incubated for 10 minutes at 56°C and to complete lysis, then invert tube 3 or 4 times throughout incubation until the red color of the lysate turns dark green indicating perfect lysis. the 1.5 ml tube was then centrifuged to remove any remaining drips from the inside of the rid.

4. Following that, 200 µl absolute ethanol was added to the lysate and thoroughly mixed with a pulse vortex. after mixing, the tube was gently centrifuged to remove drops from the inside of the cover.

5. Gently transfer the mixture to the Spin Column without soaking the rim, close the cover and centrifuge for 1 minute at 13,000 rpm. discard the filtrate and place the Spin Column in a 2 ml Collection Tube.

6. Fill the Spin Column with 700 µl of Buffer WA without wetting the rim, then centrifuge for 1 minute at 13,000 rpm. reuse the Collection Tube after discard the flow-through.

7. Centrifuged for 1 minute at 13,000 rpm after adding 700 µl of Buffer WB to the Spin Column without wet the rim, the flow-through was discarded, and the Column was placed in Collection Tube (to be reused).

to dry the membrane centrifuged for an additional 1 minute then the flow-through and Collection tube discarded .

8. Into a new 1.5 ml tube place the Spin Column and 30 - 100 µl of Buffer CE straight onto the membrane, incubate for 1 minute at room temperature then centrifuge for 1 minute at 13,000 rpm to elute.

This DNA was stable when preserved at deep freezing for a long time.

2.3.3.2. Polymerase Chains Reactions

PCR is a process that involves the replication of DNA regions using the enzyme DNA polymerase, allowing for the amplification of required DNA fragments from one molecule to many million copies. there are several requirements for a PCR reaction, including two primers that are complementary

to the target DNA, one primer binds to each side of DNA and the desired DNA sequences are amplified between them. Any PCR has three primary phases that are cycled roughly 25-45 times including: denaturation, annealing and extension (125).

2.3.3.2.A. Primers Preparation

PCR reaction was performed using a specific primer pairs designed for SCN1A c.3184 (A>G) (rs2298771) and UGT2B7 c.802 (C> T) (rs7349366) by Assistant Prof. Ahmed Abdul Jabbar Suleiman (University of Anbar ,College of science, Department of Biotechnology) using primer blast software and depending on https://www.ncbi.nlm.nih.gov/ websites. tables (2.3) and (2.4) indicate the primer sequences that were used for amplification analysis of the SCN1A and UGT2B7 genes for SNP detection.

Table (2-3): Primers sequences of SCN1A (rs2298771) c.3184 alleles(A>G)

SNPS	Sequence	Product size(bp)
Forward primer	**TCCAGATCCGATTCACTACT**	**------**
R1-Allele A	**AGTTGTGTATGTCCAATCACGT**	**239**
R2- Allele G	**AGTTGTGTATGTCCAATCACGC**	**239**

Table (2-4): Primers sequences of UGT2B7 (rs7439366) c.802 alleles (C>T)

SNPs	Sequence	Product size(bp)
O-F	**TTTGCCTACATTATTCTAACCCCTTT**	**284**
O-R	**CTGAAAATTCAAAGCCAACAAAATAA**	**284**
I-F Allele C	**AACTCCTGGAATTTTCAGTTTCAAC**	**141**
I-R Allele T	**AAATCAACATTTGGTAAGAGTGGCTA**	**194**

Lyophilized primers ordered from Bioneer, Korea, were dissolved in significant measure of nuclease free water (150 µL) according to the manufacture to obtain a concentration of 100 pmol/µl stock solution. after that, a diluted solution was formed by adding 10 µl of each stock solution primer with 90 µl of nuclease-free water to obtain 10pmol/ µl as working solution . This work solution was maintained at -20°C until it was needed.

2.3.3.2. B. Optimization of Polymerase Chain Reaction Conditions

The ideal annealing temperature and number of amplification cycles were identified after several trials of optimizing the PCR process. The optimization of SCN1A(A>G) c.3184 (rs2298771) Genetic Polymorphism of PCR conditions was prepared by using: .

- Different volumes of primers (1 µl, 1.5 µl)

- Different volumes of DNA sample (3 µl, 4 µl)
- Different annealing temperatures (55 °C, 56°C , 57 °C)

Table (2-5) Polymerase chain reaction (PCR) mix reaction for SCN1A (A>G) (rs2298771)

Component	Volume (µl)
Forward primer	1 µl
Reverse (A) primer	1 µl
Reverse (G) primer	1 µl
DNA sample	3 µl
Nuclease free water	14 µl

The optimization of UGT2B7 c.802 (C>T) (rs7439366) Genetic Polymorphism of PCR conditions were prepared by using:

- Different volumes of primers (1 µL.0.5 µL)
- Different volumes of DNA sample (3 µL,4 µL)
- Different annealing temperatures (56°C ,58 °C)

Table (2-6) Polymerase chain reaction (PCR) mix reaction for UGT2B7 (C>T) (rs7439366)

Component	Volume (µl)
outer forward primer	1 µl
outer reverse primer	1 µl
inner reverse primer	0.5 µl
inner forward primer	0.5 µl
DNA sample 4 µl	4 µl
nuclease free water	13 µl

2.3.3.2.C. Running the Polymerase Chain Reaction

The PCR reaction was done by mixing PCR components with DNA sample and primers solution using the optimized PCR programs the PCR premix- bioneer kit individual tube contains:

-Lyophilized mixture of thermostable enzyme (Taq DNApolymerase)

-DNTPs

-Reaction buffers (Tris-HCl, KCl)

-Enhancer (Mgcl2)

-Stabilizer and tracking Dye

Add only DNA template and primers

The thermal program for SCN1A (A>G) (RS 2298771) and UGT2B7 (C>T) (rs7439366) is shown in table (2-7) ,(2-8), respectively

Table (2-7): PCR Condition for genotyping for SCN1A (A>G) (rs2298771)

Steps	Temperatures/c	Time /second	Cycle
Denature template	95	3 minutes	1
Initial denaturation	95	30	33
Annealing	56	30	
Extension	72	55	
Final extension	72	5 minutes	1

Table (2-8) Polymerase chain reaction condition for genotyping of UGT2B7 (C>T) (rs7439366)

Steps	Temperatures C	Time /second	Cycle
Denature template	95	3 minutes	1
Initial denaturation	95	30	35
Annealing	58	30	
Extension	72	55	
Final extension	72	5 minutes	1

2.3.3.3. Agarose Gel Electrophoresis

a) **Prepare 1x Tris-borate EDTA (TBE) solution:** to prepare 100ml of 1x TBE adding 90 ml of Deionized water (D.W.) to 10 ml of 10x TBE buffer (1 volume of 10x TBE buffer with 9 volume of Deionized water)

b) **prepared Agarose gel:** to prepare agarose gel dissolve 1.5 g of agarose powder (1.5%) in 100 ml of 1x Tris-borate EDTA (TBE) buffer (pH = 8), heated 50 ml of the solution to boling untile dissolve all gel particles and looked pure and clear , the solution was cooled down to 50˚C and 3 µl of ethidium bromide 10 mg /ml dye were added for it.

c) **Casting agarose gel:** the comb was attached to the try about 1 inch from one ending to create holes for the samples to be loaded. Then pouring the agarose solution to tray, it was allowed to solidify at room temperature for 30 minutes. the comb was gently removed from the attempt and the gel placed in the horizontal gel electrophoresis tank

d) **Loading of PCR Products:** with great precaution 5 µl of PCR sample were directly loaded in the well and fixed in an electrophoresis chamber.

e) **DNA Ladder**: 5 µl of DNA ladder (Intron, Korea) was used with band size ladder 100-1000 bp this type can be directly offered without addition sample loading dye

f) **TBE buffer:** was used to fill this chamber until the buffer reach 3-5 mm above the surface of the gel.

g) **Electrical power**: the electrophoresis apparatus' voltage was set to 50 v and ensure an electrical field of 5 v.cm-1 for 10 cm distance between cathode and

anode. DNA moved from cathode (negative pole) to pluse anode (positive poles)

h) **Gel band Visualization:** For band detection at the end of the run, a UV transilluminator devise set to 320-336 nm was utilized. A digital camera connected to PC was used to photographed.(124,125)

2.4. Statistics Analysis

The data of participants in this study were converted into a computerized database, revised for errors or inconsistencies, and then managed, processed, and analyzed by using the statistical package for social sciences (SPSS) version 26, IBM, US.

Scale variables presented in mean, standard deviation (SD), while descriptive statistics for nominal (categorical) variables represented as frequency (number of participants) and proportion (percentage). Scales variables like Age and weight that follow the statistical normal distribution, so parametric test were applied. Student's test for two independent samples was used to compare means between groups. Analysis of variances (one way ANOVA) was used to compare more than two means. Paired t test was used to compare means at pre and post treatment within each group. The odd ratio (OR) was estimated using logistic analysis of the snp to measure response to therapy and 95 percent confidence intervals were calculated to estimate the significant of the OR .

In this study, probability value (p-value) in all statistical analysis expressed as the significant values were (<0.05). finally, results and findings were presented in tables and or figures with an explanatory paragraph for each table or figure.

Chapter Three

Results

3. Results

3.1. Demographic Characteristics of the Epileptic Patients

One hundred and thirteen patients of epilepsy dependent upon valproic acid mono therapy were enrolled in the study, after three months from baseline one hundred and three patients were continued follow up but ten patients were lost and after six months from baseline ninety patients continued and thirteen patients were follow up lost. The patients which included in the study were with age ranged between (3-52 y) and the mean of their age were 19.14 ± 13.26 and the gender distribution of patient was 57.3% male and 42.7% female as shown in figure (1-3). mean weight of patient was 51.91 ± 27.53 kg. the results of the current study showed that positive family history of the disease accounted for (45.6%) and nearly similar proportion of consanguinity (42.7%). etiology of the disease accounted for (67%) idiopathic and (25.2%) symptomatic while cryptogenic is less one accounted (7.8%) .

Simple partial seizure accounted for (41.7%) from total seizure type in the study while the least type was generalized atonic type (4.9%). onset of illness from childhood contributed nearly to one third of the study patients (31.7%). More than half of the study patients (56.6%) were not treated with any previous antiepileptic drugs (newly diagnosed), while (43.4%) treated with other AED failure treatment. table (3.1) shows the demographic data of epileptic patients of this study.

Table (3.1) Demographic characteristics of the study patients

Variable	Groups	Frequency N=113	Percentage 100%
Family history	Positive	52	45.6%
	Negative	61	54.4%
Consanguinity	Yes	48	42.7%
	No	65	57.3%
Etiology	Idiopathic	76	67%
	Symptomatic	28	25.2%
	Cryptogenic	9	7.8%
Seizure type	Simple partial	47	41.7%
	Complex partial	28	25.2%
	Generalized tonic clonic	22	19.4%
	Generalized atonic	6	4.9%
	Generalized myoclonic	10	8.8%
Onset of illness	10-18 years	24	21.4%
	5-10 years	35	30.7%
	From childhood	36	31.7%
	Adults	18	15.5%
history of other AED	Yes	49	43.4%
	No	64	56.6%

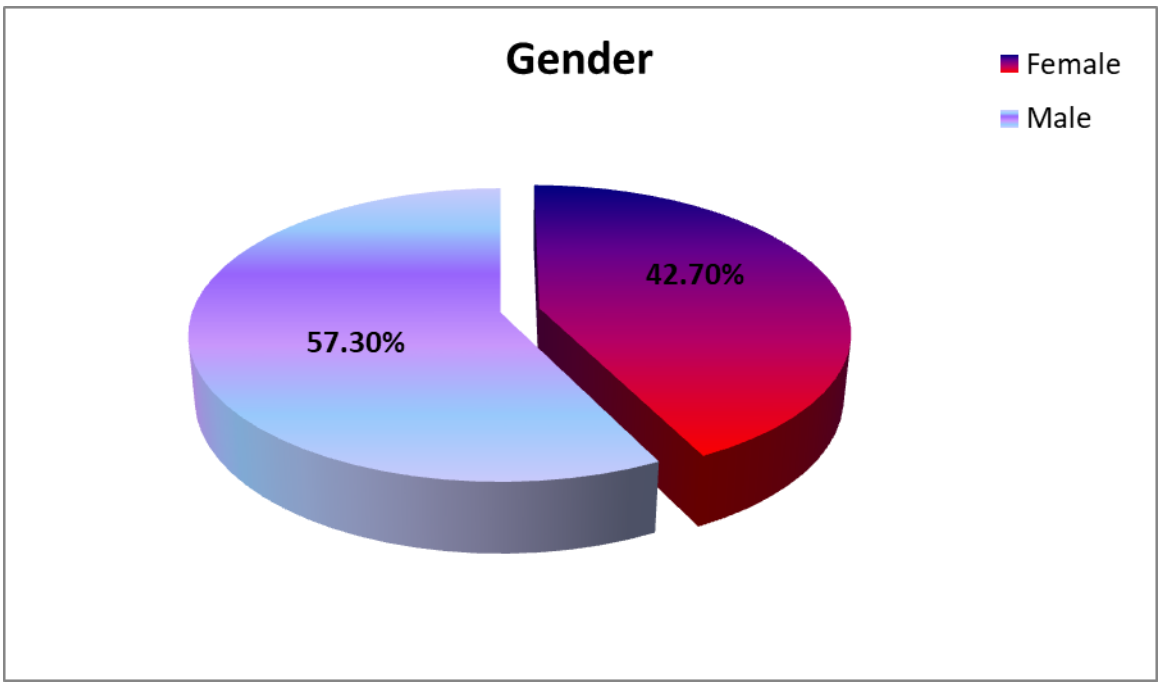


Figure (3.1) Gender percentage of the study patient

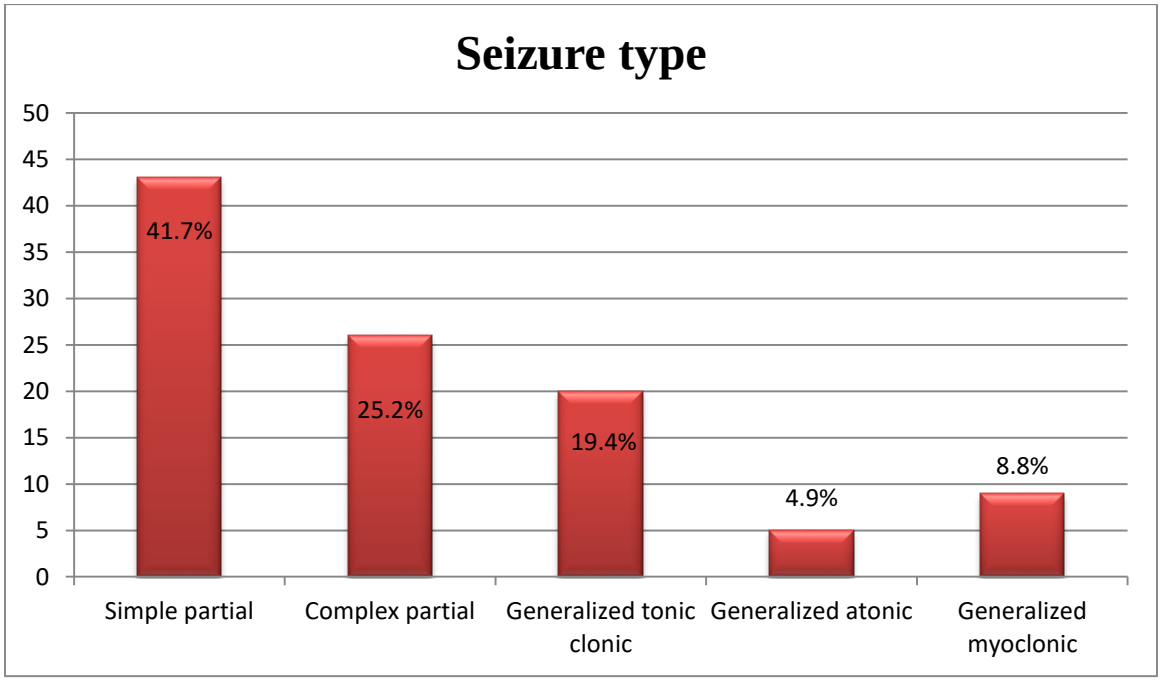


Figure (3.2) types of epilepsy enrolled in this study

3.2. Association of Demographic Characteristics of The Epileptic Patients with the Response Rate After Six Months of Treatment

Table (3-2) below reveals the association of demographic data and certain variables with the response rate. After 6 months of treatment, there are no significant statistical associations between Age, Weight, Gender, Family history , Etiology , Consanguinity, onset of illness , and VPA concentration between variables VPA- responsive group (p value >0.05) .

Whereas there were significant statistical associations in the history of AED between VPA- responsive and poor responsive group, the patients who had a history of AED were lower number and percentage 20(38.5%) in VPA-responsive group than other patients who had no history of AED with number and percentage 32(61.5%) as well as poor responsive group consist of higher number and percentage of patients who had history of previous AED response 28(73.7%) than other patients who had no history of previous AED with a number and percentage 10(26.3%) (p value 0.009).

Furthermore there were significant statistical associations of seizure types between variable responsive groups (p value 0.023). The VPA- responsive group including higher number and percentage of patients with simple partial 25(51%) as well as complex partial 13(26.5%) than other types of epilepsy. VPA-poor responsive group had near proportions of the number and percentage of the simple partial seizure 18(33.3%) ,complex partial seizure 13(24.1%) and generalized tonic clonic seizure 16(29.6%) these types were a higher number and percentage than other types in this study such as generalized atonic, generalized myoclonic 1(1.9%), 6(11.1%), respectively .

Table(3-2) Association of demographic data of the study of epileptic patients with the VPA response after six months of treatment

Variable		After 6 months of treatment N=90		
		VPA – Responsive n=52 (57.7%)	**VPA- Poor responsive n=38 (43.3%)**	**P value**
Age (y)	Mean ± ST	19.04±13.02	18.32±12.65	0.793 [NS]
Weight in Kg	Mean ± ST	51.73±25.84	51.18±28.72	0.925 [NS]
Gender	Male	30(57.6%)	22(57.8%)	0.398 [NS]
	Female	22(42.4%)	16(42.2%)	
Family history	Positive	24(46.2%)	18(47.4%)	0.909 [NS]
	Negative	28(53.8%)	20(52.6%)	
Consanguinity	Yes	24(46.2%)	18(47.4%)	0.909 [NS]
	No	28(53.8%)	20(52.6%)	
Etiology	Idiopathic	26(50%)	18(47.4%)	0.144 [NS]
	Symptomatic	24(46.2%)	14(36.8%)	
	Cryptogenic	2(3.8%)	6(15.8%)	
Seizure type	Simple partial	25(51%)	18(33.3%)	**0.023 [S]**
	Complex partial	13(26.5%)	13(24.1%)	

	Generalized tonic clonic	4(8.2%)	16(29.6%)	
	Generalized atonic	4(8.2%)	1(1.9%)	
	Generalized myoclonic	3(6.1%)	6(11.1%)	
Onset of illness	10-18years	12(24%)	8(21.1%)	0.981 [NS]
	5-10 years	8(16%)	6(15.8%)	
	From childhood	14(28%)	12(31.6%)	
	Adult	16(32%)	12(31.6%)	
History of AED	Yes	20(38.5%)	28(73.7%)	**0.009 [S]**
	No	32(61.5%)	10(26.3%)	
VPA concentration Mean ± SD	After 3 months	52.89 ± 23.46	46.76 ± 20.68	0.163 [NS]
	After 6 months	55.01 ± 16.85	58.62 ± 10.03	0.241 [NS]

3.3. Valproic Acid Dose and Concentration of the Epileptic Patients

Table (3-3) below shows all the enrolled patients who were treated with valproic acid (10-15 mg/kg) monotherapy that the mean VPA initial dose /day at baseline was 515.53±289.61 on the basis of their seizure control at the baseline ,patients were evaluated at the 3rd month of the VPA therapy for clinical

scoring to assess epilepsy management and their mean ±SD of VPA concentration were 49.68 ± 22.25. patients with poor seizure control and who had low VPA concentration than normal (therapeutic level 50-100 µg/ml) were dose escalated and mean maintenance dose became 599.03 ± 330.33 , whereas after 6 months 669.9 ± 366.44 is a mean ±SD of the dose after subsequent increment or decrement with VPA concentration mean ± SD 56.53±14.41

Table(3-3): Dose and concentration of VPA treatment in the study patients

Variables	N	Minimum	Maximum	Mean	Std. Deviation
VPA initial dose /day at baseline	113	100 mg	1000 mg	515.53mg	289.61
VPA maintenance dose at first 3 months	103	100 mg	1500 mg	599.03mg	330.33
VPA concentration after 3 months	103	15.8 µg/ml	120.56 µg/ml	49.68 µg/ml	22.25
Dose after subsequent increment or decrement if found after 6 months	103	100 mg	1500 mg	669.9 mg	366.44
VPA concentration after six months	90	35.79 µg/ml	95.55 µg/ml	56.53 µg/ml	14.41

3.4. Association of Clinical Parameters of the Epileptic Patients with the Response Rate After Three and Six months from Valproic Acid Treatment

The efficacy measures included the percentage change of seizure frequency depending on registered numbers of seizure attacks of any type per

month from baseline through the treatment period. Patients were divided into two groups based on their responsive to valproic acid therapy over three months, and six months VPA-responsive patients had response rate ≥ 50% reduction in number of seizure attacks from baseline and VPA- poor responsive patients had response rate <50% reduction in number of seizure attacks from baseline.

As the table (3-4) after three months of treatment and based on number of seizures attacks per month which recorded in patients card ,49 patients had good response to valproic acid treatment with response rate of ≥50% reduction in seizures attack from baseline and had significant statistical association between the frequency of seizure attack per month before treatment (mean ±SD 3.08 ± 2.07) and frequency of seizure attack per month after 3months of treatment (mean ±SD 1.2 ± 1.8). while 54 patients were found with poor response to valproic acid treatment with response rate of <50% reduction in seizures attack from baseline and had significant statistical association between the frequency of seizure attack per month before treatment (mean± SD 2.95 ± 2.2) and the frequency of seizure attack per month after 3months of treatment (mean± SD 2.1 ± 1.8) p value is (**< 0.001 , < 0.001** respectively).

After six months of treatment, 52 patients were found with good response to valproic acid with response rate of ≥50% reduction in seizures attack from base line therapy of valproic acid and had significant statistical association between frequency of seizure attack per month before treatment with (mean ± SD 2.26 ± 1.98) and after six months of treatment (mean ± SD 1.01 ± 2.13). While 38 patients were found with poor response to valproic acid with response rate <50% reduction in frequency of seizures attack from baseline and had significant statistical association between the frequency of seizure attack per

month before treatment (1.78 ± 1.78) and after six months of treatment (1.42 ± 1.85) (p value < 0.001**,** 0.001 respectively)

Table (3- 4) Association between the frequency of seizure attacks /month at baseline , after three months and after six months of treatment

	Response rate	Frequency of seizure/month at baseline	Frequency of seizure/month after treatment	Mean difference ±SD	P value
After three months	VPA-Responsive N=(49)	3.08 ± 2.07	1.2 ± 1.8	1.88±0.27	**< 0.001 [S]**
	VPA Poor-responsive N=(54)	2.95 ± 2.2	2.1 ± 1.8	0.85±0.4	**< 0.001[S]**
After six months	VPA-Responsive N=(52)	2.26 ± 1.98	1.01 ± 2.13	1.25±0.15	**< 0.001[S]**
	VPA Poor-responsive N=(38)	1.78 ± 1.78	1.42 ± 1.85	0.36±0.07	**0.001[S]**
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant					

3.5. Genetic Analysis of Detected Genotypes of SCN1A c.3184 (A>G) (rs2298771) and UGT2B7 c.802 (C> T) (rs7439366) in the Iraqi Epileptic Patients

The amplification of SNP of SCN1A gene (A>G) (rs2298771) was shown in bp239 as in figure (3.3 A and B) and amplification of SNP of UGT2B7 gene (C> T) (rs7439366) was shown in bp141,bp 194 as in figure (3.4)

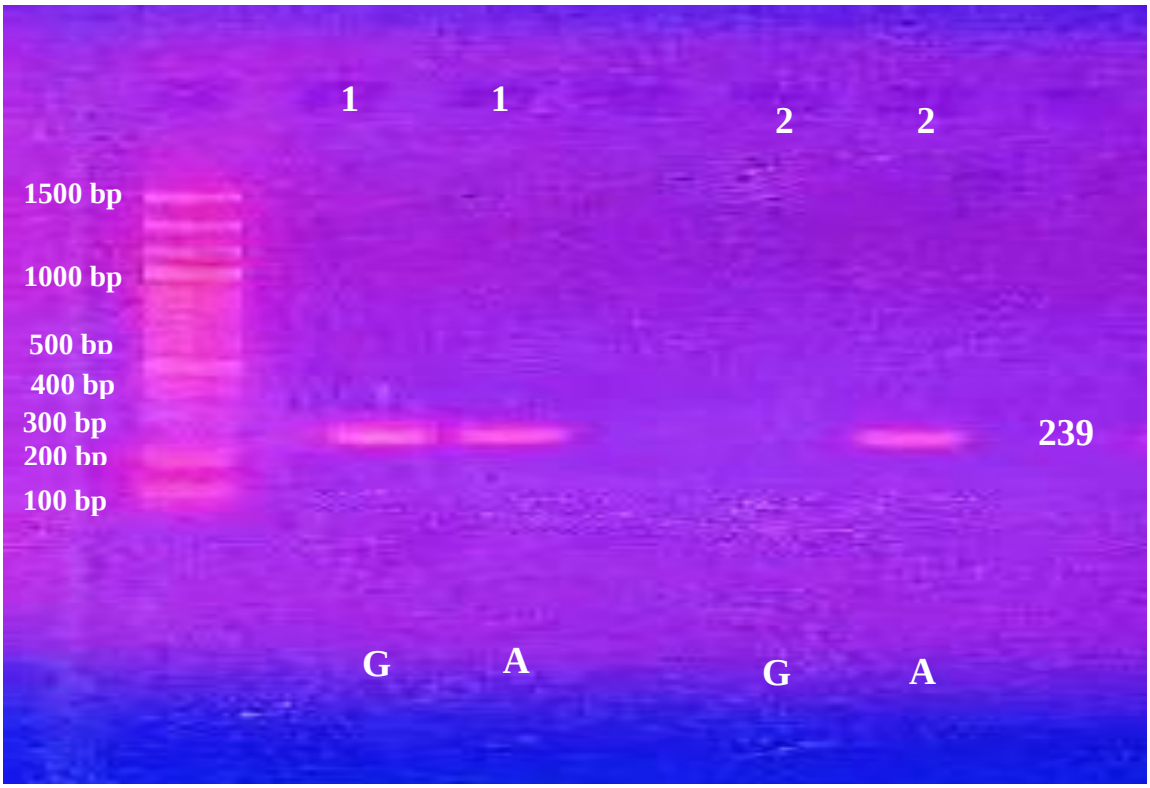


Figure (3.3.A): The Allele Specific PCR (AS-PCR) of SCN1A gene: (A>G) (rs2298771) genetic polymorphism showed: this picture represented two samples, lane M represented DNA ladder (100-1500bp), 1st sample represented hetrozygous (AG) genotype was shown in (239bp), 2nd sample: represented homozygous (AA) genotype was shown in (239bp).

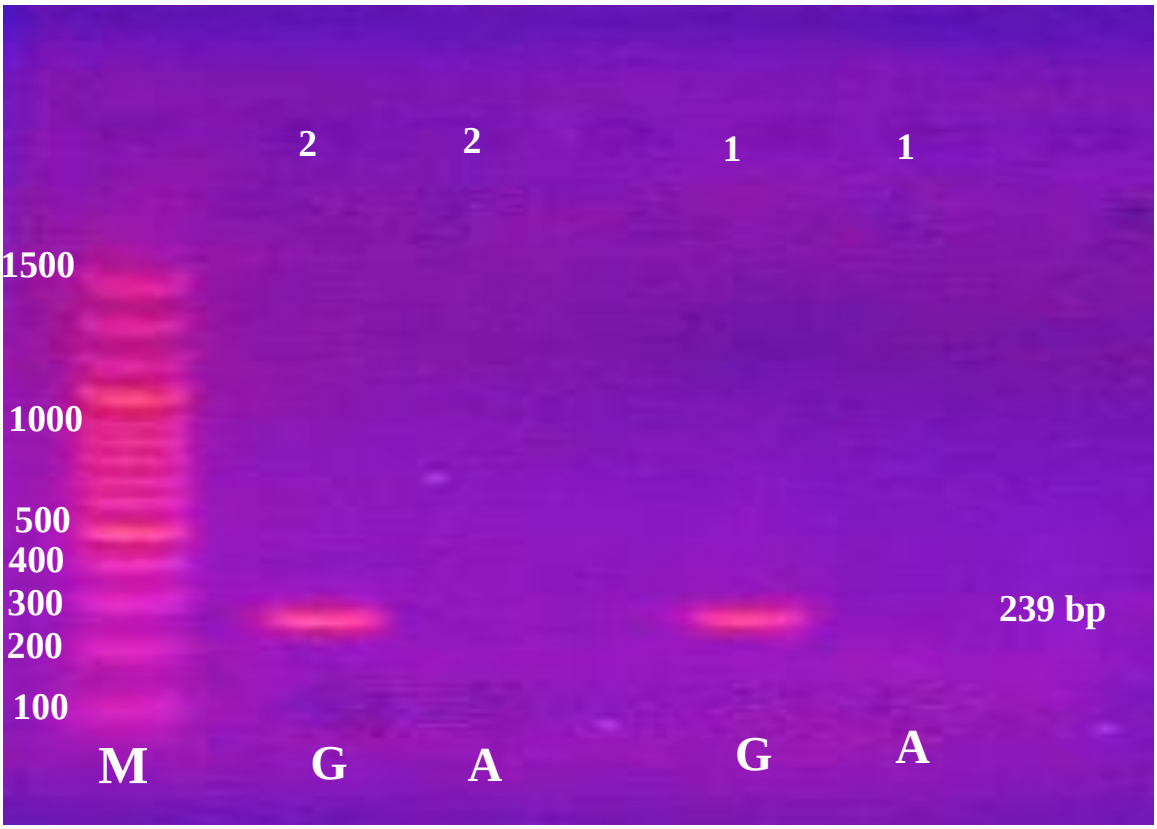


Figure (3.3.B): The Allele Specific PCR (AS-PCR) of SCN1A genec.3184 (A>G) (rs 2298771) genetic polymorphism showed: this picture represented two sample, lane M represented DNA ladder (100-1500bp), 1st and 2nd sample represented homozygous (GG) genotype was shown in (239bp).

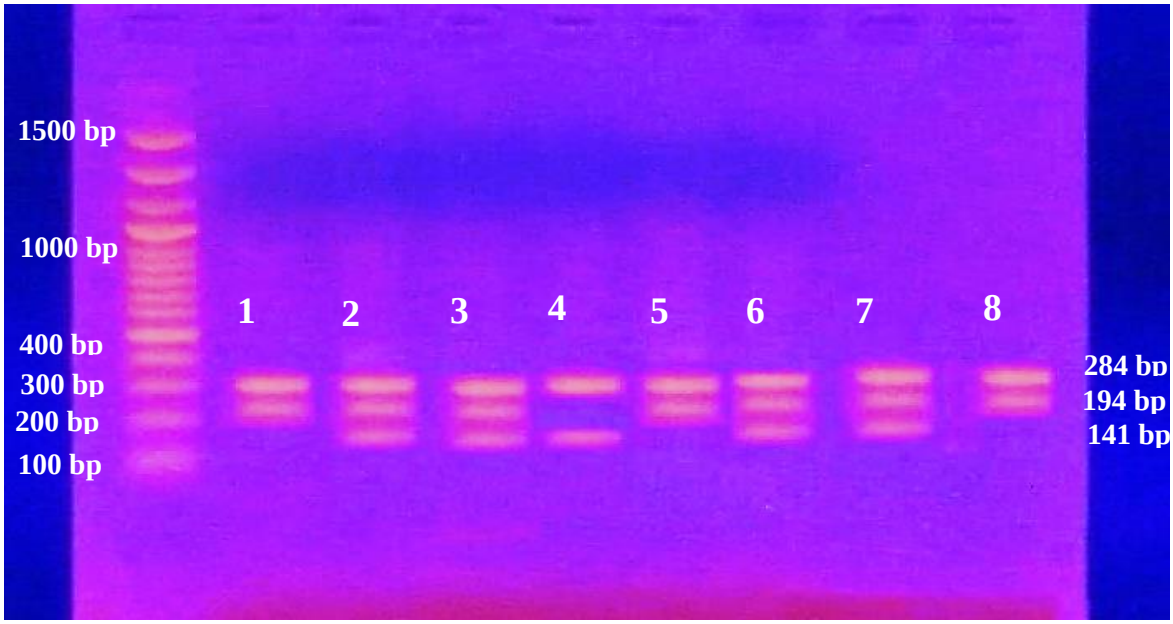


Figure (3.4): The amplification refractory mutation system (ARMS-PCR) of UGT2B7 c.802 (C>T) (rs7439366) genetic polymorphism showed: lane M: represented DNA ladder (100-1500 bp), lane 1,5and 8: represented mutant genotype (TT), lane 4: represented wild genotype (CC) and lane 2,3,6,and 7: represented mutant type heterozygous (CT).

3.6. The Frequency and Percentage of Detected Genotypes of SCN1A c.3184 (A>G) (rs2298771) and UGT2B7 c.802 (C> T) (rs7439366) in the Iraqi Epileptic Patients

The patients enrolled in the present study were classified according to their genotypes for SCN1A c.3184 (A>G) (rs2298771) genetic polymorphism , the most frequent genotype of SCN1A c.3184 (A>G) (rs2298771) detected in 113 epileptic patients in this study was (AA) with frequency and percentage of 49 (43.7%) respectively, which is considered a major type while the frequency and percentage of homozygous minor type (GG) was (25.2 %) and heterozygous type (AG) was (31.1 %) as in the table (3.5).

Table (3.5): Distribution of detected genotypes of SCN1Ac.3184 (A>G) (rs2298771) among the study epileptic patients

SNP	Genotypes	Frequency	Percentage
SCN1A genotype (A> G) (rs2298771)	AA	49	43.7 %
	AG	35	31.1 %
	GG	29	25.2 %
	Total	113	100 %

For of UGT2B7 c.802 (C> T) (rs7439366) , the mutant genotype (TT) was the most frequent genotype which was detected in a higher number and percentage 65(58.2%) ,wild (CC) was detected in low frequency and percentage (17.5 %), another genotype also detected (CT) (24.3 %) which was heterozygous, as in table (3.6).

Table (3.6): Distribution of detected genotypes of UGT2B7 c.802 (C> T) (rs7439366) in the epileptic patients

SNP	Genotypes	Frequency	Percentage
UGT2b7 genotype UGT2B7 (C> T) (rs7439366),	CC	20	17.5 %
	CT	28	24.3 %
	TT	65	58.2 %
	Total	113	100 %

3.7. Association of Genotype SCN1A c.3814 (rs2298771) Polymorphism in Epileptic Patients with Clinical parameter and Response Rate After three and Six Months of Valproic Acid Treatment

A. After 3 months of valproic acid therapy,

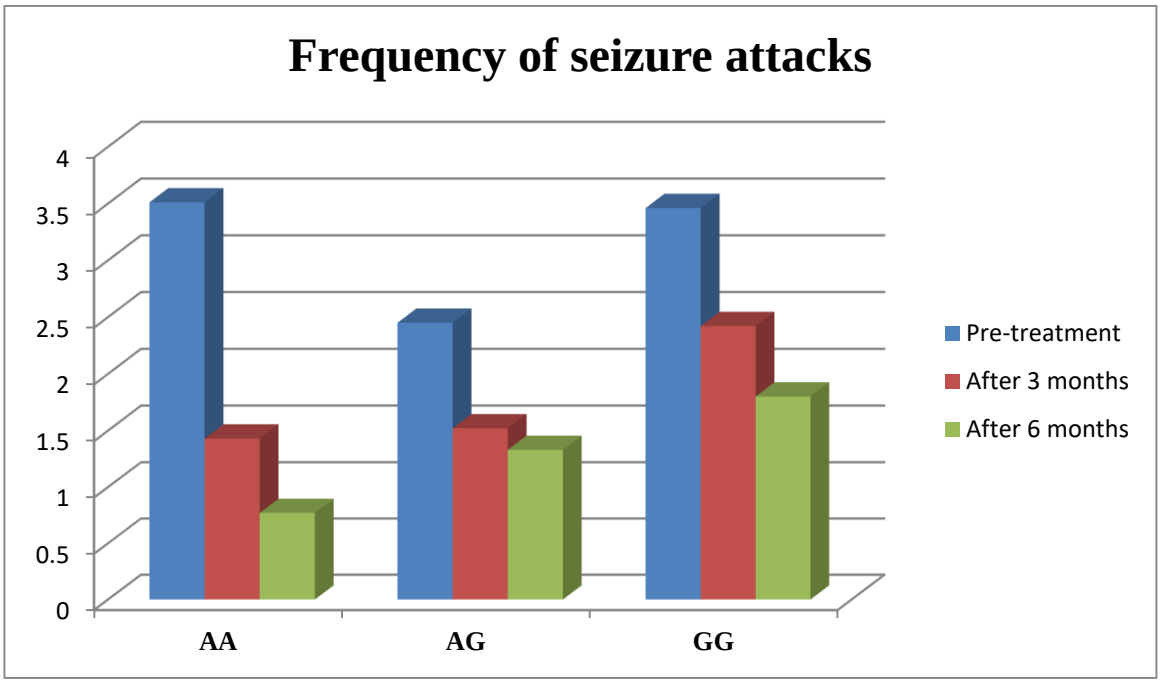
Table (3-7) shows the AA genotypes carriers had significant lower seizure attacks with a rate more than 50 % from baseline while GG and AG genotypes carrier had seizure attacks were lowered significantly less than 50 % from baseline after three months of valproic acid mono-therapy as in the figure (3-5) . Furthermore results in table (3-8) revealed significant differences in three allele groups between difference responsive group, ($p < 0.05$). It has been observed that AA carriers were significantly greatly higher in number and percentage in VPA-responsive patients 36 (73.5%)compared to AG and GG carriers with number and percentage 9 (18.3%) ,4 (8.2%) respectively , while poor-responsive group had AG carriers and GG carriers with a significantly higher number and percentage 23 (42.6%) 22 (40.7%) respectively, than AA carriers with number and percentage 9 (16.7%) P value < 0.001

B. After 6 month of valproic acid therapy

Table (3-7) shows the AA genotypes carrier had significant lower of the seizure attacks with a rate more than 50 % from baseline while as GG and GA genotypes carrier the frequency of seizure attacks were lowered significantly with rate less than 50 % from baseline as in the figure (3-5).

Table (3-7) Association of SCN1A c.3184 (rs2298771)A>G genotypes with the frequency of seizure attacks before, after 3 and 6 months of treatment

SCN1A genotypes	mean of attacks/ month pre treatment	mean of attacks/ month after 3 months	mean of attacks/ month after six months	P value
AA	3.51± 2.2	1.43± 1.88	0.77± 0.6	0.029**[S]**
AG	2.45± 1.5	1.52± 1.4	1.33± 0.15	0.001**[S]**
GG	3.46± 2.8	2.42± 2.14	1.8± 1.2	0.003**[S]**
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant				



(Fig. 3-5) Association of SCN1A c.3184 (rs2298771) A>G genotypes with the frequency of seizure attacks before, after 3 and 6 months of treatment

Furthermore results in table (3-8) revealed significant differences in three alleles between different responsive groups, ($p < 0.05$). It has been observed that VPA-responsive patients had AA carriers significantly higher in number and percentage 36 (69.2%) compared to AG and GG carriers with numbers and percentages 14 (27%) ,2(3.8%) respectively , while poor-responsive groups had GG carriers with significantly higher numbers and percentages 20 (52.6%), than AG carriers and AA carriers with number and percentage 12(31.6%) 6 (15.8%), respectively. P value < 0.001

Table (3-8) Association of SCN1A c.3184 (rs2298771) A>G genotypes with response rate after 3 and 6 months of treatment

After three months			
Genotypes of SCN1A (rs2298771)A>G	VPA-Responsive	VPA- poor Responsive	P value
GG	4 (8.2%)	22 (40.7%)	**< 0.001[S]**
AG	9 (18.3%)	23 (42.6%)	
AA	36 (73.5%)	9 (16.7%)	
Total n= 103	N=49	N=54	
After six months			
GG	2(3.8%)	20 (52.6%)	P value
AG	14 (27%)	12(31.6%)	**< 0.001[S]**
AA	36 (69.2%)	6 (15.8%)	
Total n= 90	N=52	N=38	
Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant			

Table (3-9) illustrate the epileptic patients' response to valproic acid therapy according to logistic regression analysis and by using the AA allele as reference there was a significant difference in response to valproic acid between the GG and AA and between AG and GG alleles in which the GG and AG have a lower incidence of response as compared to AA allele after three and six months of the therapy

Table (3-9): logistic regression analysis of SCN1A c.3184 (rs2298771)A>G genotypes with response rate at 3 and 6 months of treatment with VPA.

	Genotype	**OR (95% CI)**	**p value**
After 3 months	**GG**	0.045 (0.012-0.165)	**0.001[S]**
	AG	0.098 (0.034-0.283)	**0.001[S]**
	AA	1[a]	-
After 6 months	**GG**	0.017 (0.003-0.09)	**0.001[S]**
	AG	0.194 (0.061-0.619)	**0.006[S]**
	AA	1[a]	-
p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, a; reference category			

3.8. Association of UGT2B7 c.802 C>T (7439366) Genetic Polymorphism with Valproic Acid Concentration and Response After Three and Six Months of Treatment

All patients diagnosed with epileptics with normal liver function, were treated with VPA as monotherapy in regularly administration to assure the steady-state concentrations were achieved. Mean ±SD of VPA dose after three and six months of treatment was 599.03±330.33 and 669.9 ± 366.44 respectively.

Blood sampling was performed within 1 hour prior to a scheduled dose trough concentration was measured by direct chemiluminescence assay with a linear range of (2 -150 μg/ml), mean±SD of VPA concentration after three and six months of treatment was 49.68±22.25 and 56.53 ± 14.41 respectively.

The VPA plasma concentration at steady-state was adjusted by body weight and dosage of each patient and expressed as AC (adjusted concentration) AC (μg /mL per mg/kg) with mean ±SD 4.6 ± 1.81 and 5.1 ± 1.73 after three and six months respectively.

To explore effects of the genetic factors on interaindividual variations in VPA Bioavailability of epilepsy patients, table (3-10) below shows that there were significant statistical associations between valproic acid concentration and UGT2B7 polymorphism of three alleles. after 3 and 6 months of treatment Post hoc tests revealed that the adjusted serum concentration of VPA was much lower in those patients with a T allele at UGT2B7 C802T than in those with the CC genotype after three and six months, respectively.

Table (3-10) Association between UGT2B7 c.802 C>T (rs7439366) polymorphism and adjusted concentration (AC) of valproic acid

	UGT2B7 c.802 C>T (rs7439366)			**p value**
	CC	**CT**	**TT**	
VPA (AC), [(µg/mL)/(mg/kg) After 3 months	5.83±1.9	3.94±2.13	4.13±1.41	**0.001 [S]**
VPA (AC), [(µg/mL)/(mg/kg)] After 6 months	6.78±1.91	4.35±1.94	4.17±1.35	**< 0.001 [S]**
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant				

Furthermore this study investigated the relationship between UGT2B7 polymorphism and rate of VPA response. the results shows that there was no significant deference in number and percentage of CC, CT ,TT genotypes carriers between responsive and poor responsive group after three and six months of VPA treatment.(p value 0.684 , 0.148 respectively) as in the table (3-11)

Table (3-11) Association between UGT2B7 c.802 C>T (rs7439366) polymorphism and VPA response after three and six months of VPA treatment

After three months			
UGT2B7 genotype	**Groups**		**p value**
	VPA-Responsive	**VPA-poor responsive**	
CC	7(14.3%)	11(20.4%)	0.684 [NS]
CT	13(26.5%)	12(22.2%)	
TT	29(59.2%)	31(57.4%)	
Total N=103	N= 49	N= 54	
After six months			
CC	8(15.4%)	6(15.8%)	0.148 [NS]
CT	14(26.9%)	4(10.5%)	
TT	30(57.7%)	28(73.7%)	
Total N=90	N=52	N=38	
Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [NS]= Non significant			

Table (3-12) illustrate the epileptic patients' response to valproic acid therapy, according to logistic regression analysis and by using the TT allele as reference there was a no significant difference in response to valproic acid between the CC and TT and between CT and TT alleles.

Table (3-12): Logistic regression analysis of UGT2b7 (C> T) (rs7439366) genotypes with response rate at 3 and 6 months of treatment with VPA.

	UGT2b7 genotype	**OR (95% CI)**	**p value**
After 3 months	**CC**	0.68 (0.232-1.992)	**0.482[NS]**
	CT	1.158 (0.455-2.964)	**0.758[NS]**
	TT	1[a]	**-**
After 6 months	**CC**	1.244 (0.383-4.038)	**0.716[NS]**
	CT	3.267 (0.96-11.117)	**0.058[NS]**
	TT	1[a]	**-**
p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, a; reference category			

3.9 Association of UGT2B7 c.802 C>T (rs7439366) Polymorphism with Liver Enzyme pre and post 6 months of VPA Treatment and Susceptibility to Hepatotoxicity

The analysis of the data of the current study revealed that the associations of mean ± SD levels of hepatic enzymes after valproic acid treatment according to UGT2B7 polymorphism were demonstrated in (table 3-13), where AST, ALP, total bilirubin level was significantly elevated (within normal value) after treatment in patients with CC genotype (P value = 0.001 , 0.004 , and 0.004 respectively). Also total bilirubin was significantly elevated (within normal value) after treatment in patients with CT genotype , (P. value

=0.004). AST, ALP had no significant association in CT carrier .AST, ALP in addition to total bilirubin had no significant association with TT genotypes carrier.

Table (3-13) Association between UGT2B7 c.802 C>T (rs7439366) polymorphism and liver enzymes pre and post treatment with VPA

UGT2B7 genotypes		AST		ALT		Total Bilirubin	
		pre	**Post**	**Pre**	**Post**	**Pre**	**post**
CC (n=14)	***mean ± SD***	22.44 ± 6.86	31 ± 12.59	19.33 ± 5.22	24.67 ± 8.94	0.33 ± 0.25	0.51 ± 0.18
	Statistical test	t-test (paired) = 4.04		t-test (paired) = 3.37		t-test (paired) = 4.35	
	P value	**0.001**		**0.004**		**0.001**	
CT (n=18)	***mean ± SD***	22.14 ± 4.45	43.43 ± 23.43	15.43 ± 2.14	31.71 ± 18.9	0.4 ± 0.12	0.6 ± 0.32
	Statistical test	t-test (paired) = 1.95		t-test (paired) = 2.12		t-test (paired) = 3.48	
	P value	0.074		0.053		**0.004**	
TT (n=58)	***mean ± SD***	22.83 ± 7.62	22.59 ± 7.53	19 ± 6.72	18.86 ± 6.75	0.49 ± 0.34	0.54 ± 0.24
	Statistical test	t-test (paired) = 0.504		t-test (paired) = 0.27		t-test (paired) = 1.73	
	P value	0.616		0.786		0.089	

Chapter Four

Discussion

4.Discussion

Epilepsy is a brain disorder that is characterized by an enduring predisposition to produce epileptic seizures, which are transient occurrences of signs and/or symptoms caused by abnormal excessive or synchronous neuronal activity in the brain, as well as the neurobiologic, cognitive, psychological, and social consequences of this condition (126). Despite advancements in pharmacological treatment of epilepsy, pharmacoresistance remains a challenge. Understanding the pharmacogenetic reasons is essential for predicting drug response and thus providing a basis for individualized treatments. Pharmacogenetics is the study of how genes influence drug response, which can range from a lack of the desired therapeutic effect to develop an adverse drug reaction. Genetic changes in the function of drug target and drug metabolizing proteins could explain the emergence of the pharmacoresistant (127).

Valproic acid (VPA; 2-propylpentanoic acid) is an antiepileptic agent with a narrow therapeutic range (50–100 μg/mL) in the treatment of epilepsy and significant individual variability in both its pharmacokinetics and pharmacodynamics. Drug resistance is complicated, and mounting research suggests that genetic variants can influence an individual's response. Although the specific mechanism underlying this individual variability are elusive, two factors are thought to be involved in VPA's therapeutic effect: steady-state target site concentration and target receptor function. VPA steady-state concentration at target site is influenced by genetic differences in its absorption, metabolism, and transport across the blood-brain barrier. Target receptor function is altered by genetic variations in its structure, and it may be one of the key factors of individual phenotypic variances in responsiveness to AEDs, therefore the

efficacy of valproic acid (VPA) varies greatly in clinical treatment of epileptic patients (128).

This study aims to explore the possibility of a relationship between SCN1A c.3184 A>G rs2298771 and UGT2B7 c.802 C>T rs7439366 polymorphisms with VPA response via determinant variability in VPA pharmacokinetics and pharmacodynamics. Two single nucleotide polymorphisms (SNPs) in two potential genes related to receptor and drug-metabolizing enzyme were genotyped.

SCN1A encodes the Nav1.1 channel subtypes, which are the primary sodium channels in the central nervous system (CNS). SCN1A which encodes the α subunit of the Nav1.1 channel, is the gene most frequently linked to epilepsy. Mutations in SCN1A cause a wide variety of severities of hereditary epilepsy (20). Nav1.1 is expressed mostly in GABAergic neurons SCN1A genetic variations may be major factors of individual phenotypic variances in responsiveness to AEDs, which has aroused the interest of researchers (129).

The present study investigates the effect of SCN1A c.3184 A>G (rs2298771) gene polymorphisms on VPA efficacy in the Karbala epileptic population, which may be an important determinant of individual variability in VPA pharmacodynamics and it attempted to provide genetic evidence for individualized VPA treatment by detecting specific gene that can predict VPA treatment resistance.

UGT2B7 is a key enzyme in the 50% of VPA metabolism. c.802 C>T is one of UGT2B7's functional mutations may be one predictor of individual variability in VPA pharmacokinetics, and VPA dose in individuals should be adjusted to the therapeutic range of 50–100 μg/ml to assure achievement (75)

The current study evaluated the effect of the UGT2B7 c.802 C>T (rs7439366) polymorphism on VPA serum concentrations and the effect of this polymorphism on VPA therapeutic responsiveness in the Karbala epileptic population.

Hepatotoxicity is a reversible or irreversible adverse effect related with the use of valproic acid (VPA) in the treatment of epilepsy. the development of VPA reactive metabolites, inhibition of fatty acid oxidation, excessive oxidative stress, and genetic variations of certain enzymes, such as UGTs and CYPs genes, have all been linked to VPA hepatotoxicity (130).

This study investigated the effect of the UGT2B7 C802T rs7439366 polymorphisms on serum liver enzyme in patients with normal liver function (pre-treatment) and receiving valproic acid monotherapy for 6 months to discover genetic risk factors for VPA-induced hepatotoxicity in the Karbala epileptic population. .

To date, this is the first study in Iraq to examine the contribution of both genetic variants of SCN1A c.3184 A/G rs2298771 and UGT2B7 C802T rs7439366 polymorphisms with valproic acid response variation and susceptibility to VPA-induced hepatotoxicity among Iraqi epileptic patients.

4.1. Demographic Characteristics of the Epileptic Patients

Epilepsy is a multifactorial disease, with various causes contributing to its occurrence. Besides genetic predisposition, environmental factors, demographic features and other factors may all play a role in the incidence of epilepsy (131). In this study, the mean age of epileptic patients was 19.14 ± 13.26, some of them had new or delayed in diagnosis without treatment, and other had previously ineffective therapy.

According to the current study results in the gender distribution of the patients, epilepsy was a higher frequent in male than female epileptic patients with a frequency (57.3%) and (42.7%) respectively as a shown in the figure (3-1). There is wide agreement among studies indicating females have a considerably lower incidence of epileptic and unprovoked seizures than males. This different is usually related to males' higher exposure to risk factors for lesional epilepsy and acute symptomatic seizures (132).

The first step in the diagnosis of epilepsy is a questionnaire that identifies the patients' family history of epilepsy. However, because of the heterogeneity of epilepsy, the impact of epilepsy family history on epilepsy classifications, etiology, patient demographics, and epilepsy investigations remains unclear and probable complex (133), seizures in first-degree relatives are usually a significant indicator of mostly hereditary epilepsies. In addition, the presence of a family history of epilepsy was associated with a higher risk of seizure recurrence after a single seizure or febrile convulsion (134,135) and may underlie the possibility of a genetic etiology which could be related to population's high incidence of consanguinity. According to the findings of the current study, a positive family history of the disease accounted for (45.6%) of

the percentage and a nearly similar proportion of the percentage of consanguinity (42.7%). These findings are consistent with a study conducted in Saudi Arabia, which found a family history of epilepsy in large portion of the patients studied (136) , as well as a greater rate in other countries such as Turkey (137,138) This could be related to the high rate of consanguineous marriage observed in those regions, including Iraq.

The etiology of epilepsy that occurs as a consequence of the causes, was idiopathic, symptomatic, cryptogenic epilepsy. The causes of idiopathic epilepsy are predominantly genetic, with early onset during childhood, whereas symptomatic epilepsies are caused by a brain insult. on the other hand, cryptogenic epilepsies are unknown causes. recent advances in genetic analysis have revealed that most forms of epilepsy are caused by a combination of genetic and acquired causes (139) .The demographic data of this study shown the etiology of the disease accounted for 67% idiopathic and 25.2% symptomatic while cryptogenic is less one accounted 7.8%. The existence of such a large sample of epileptic patients with idiopathic epilepsy in a region with a high frequency of consanguineous marriage enhanced the ability to explore how the family history of epilepsy impacts the etiology of patients with epilepsy in this study population.

The age of epilepsy onset, defined as the age at which the first seizure occurs, the onset of illness in this study shows the onset of epilepsy in childhood is higher in number and percentage than other age category 32 (31.7%), the fact of that was the many children in Iraq might be born with a genetic defect, and they are more likely to be influenced by environmental variables such as vehicle accidents, head trauma, brain tumors, and infectious diseases, in addition to the consequences of wars (140). furthermore that some study have elucidated that

the rate of occurrence varies wildly depending on age is greatest during the first year of life and early childhood, followed by adolescence (145). It declines between the ages of 20 and 40, then gradually increases after 50, with peoples over the age of 80 having the greatest increase (10).

Classification of epileptic types is according to clinical data obtained by medical history, along with EEG and MRI results and according to the International League against Epilepsy has recently adjusted its classification of seizures and epilepsies into generalized, partial , or unclassified (or unknown) (117). valproic acid is wide spectrum effectiveness against all the types of seizure and epileptic syndromes, in both pediatric and adult patients ,it has been evaluated in both generalized (tonic-clonic, absences, and myoclonic) and focal seizures and represents one of the most effective antiepileptic drugs (AEDs in all types of epilepsy) (67) . valproic acid was administered to all epilepsy types were enrolled in this study, with outcomes assessed after three and six months of treatment, the distribution consisting of 41.7% of the patients suffering from simple partial seizures, 25.2% of the patients suffering from complex partial seizures, and 19.4% of the patients suffering from generalized tonic-clonic seizures, 8.8% are generalized myoclonic and 4.9% are generalized atonic.

4.2. Association of Demographic Characteristics of The Epileptic Patients with the Response Rate After Six Months of Valproic Acid Treatment

The study hypothesis was that the association of gene polymorphism with drug-response could be masked by the differential number of factors known to influence epilepsy drug-response. As a result, it is essential to investigate the relationship between polymorphisms and treatment response, taking into account and classifying individuals based on demographic characteristics that influence drug- response in epilepsy.

The sample population was followed up after three and six months In reality, the demographic features of 90 patients after six months of valproic acid treatment there were not significant variations between the VPA-responsive and VPA-poor response groups in the age, gender, weight, family history consanguinity, epilepsy etiology, onset of disease, and VPA concentration after six months of valproic acid treatment are shown in the table (3-2), another study as well founded no significant difference between the VPA-responsive and VPA-resistant groups in characters :age, gender, epilepsy etiology, onset of disease (29).

While there is a significant difference in types of epilepsy between the VPA-responsive and VPA-poor responsive groups, the number and percentage of patients with simple partial and complex partial epilepsy 25(51%),13(26.5%) respectively, was a greater than other epilepsy types in the VPA responsive group and as well as in the poor responsive group the frequency and percentage of simple partial and complex partial epilepsy generalized tonic clonic epilepsy was 18(33.3%) 13(24.1) 16(29.6%) respectively , which higher numbers and

percentages of epilepsy types in VPA poor responsive group (p value is 0.023) Although potential risk factors for drug-resistant epilepsy are known, it has not yet been explained why in two patients with the same type of epilepsy or the same type of seizures, the effectiveness of treatment with antiepileptic drugs can be extremely different (51), the probable causes of this phenomenon are genetic factors responsible for changing the pharmacodynamic and pharmacokinetic properties of the drugs used. The group of these factors includes: genetically determined polymorphism of some microsomal enzymes , drug transport proteins **(**P glycoproteins), or disorders of pharmacodynamic function of neurotransmitter gamma-aminobutyric acid (GABA) receptors or mutations in the genes encoding voltage-gated ion channel subunits (143,144) . valproate is an important monotherapeutic agent that is greatly effective in the treatment of generalized epilepsies and partial seizures that do not generalize. According to comparative studies, valproate is at least as effective in the treatment of generalized and partial seizures as phenytoin and carbamazepine (145). a previous study reported there was a significant association between drug resistance and covariates such as type of seizures in their study (146)

Furthermore, there is a significant association of history of AED between the VPA-responsive and VPA-poor responsive groups (p values 0.009). Patients having a history of previous antiepileptic drugs were found in a lower number and percentage in the responding group, and higher number and percentage in the poor responsive group. This result was consent with another study which reported that the response to newly administered AED treatments was heavily influenced by previous treatment history. Seizure-free rates fell after previous AEDs proved ineffective (147).

4.3. Valproic Acid Dose and Concentration of the Epileptic Patients

All enrolled patients were treated with valproic acid (10-15 mg/kg) (148) monotherapy based on seizure control, so the mean dose ±SD in the baseline is 515.53± 289.61. In epilepsy treatment, therapeutic drug monitoring is recommended to ensure that AED serum concentrations remain within the therapeutic window (149) , An adequate VPA concentration is required for antiepileptic efficacy ,therefore TDM was included in this investigation. Following three and six months of valproic acid therapy, 103 patients were followed up. after three months, 54 patients with poor seizure control were escalated dose to 15mg/kg , taking serum valproic acid concentration in this group into account. the patient with low serum therapeutic level mean ± SD (46.76 ± 20.68) had the dose increased to maximum tolerated dose mean± SD (599.03 ± 330.33).

The Variations in VPA concentration levels were not significant in responders and poor responders during the third and sixth months of therapy (p value is 0.163,0.241 respectively) The effects of a specific serum concentration can differ between individuals; generally, clinical efficacy is expected when drug levels in the steady state are within the therapeutic range . In the present study, therapeutic steady-state valproic acid levels did not establish successful clinical drug response, After six months of examination, the total number of patients is 90, the most of the responsive patients (52 patients) had valproic acid levels above the minimal effective concentration mean± SD (55.01± 16.85); however, this did not imply that all patients with therapeutic steady state drug levels in this study were responsive. however 38 of them remain resistant to

valproic acid despite of serum VPA concentrations in the therapeutic range mean± SD (58.62 ± 10.03) as in the table (3-2). Furthermore, some patients in the VPA poor responsive had levels that above 100 mg/ml they had levels ranging from 100 to 120 g/mL, and these patients displayed indications of valproic acid toxicity. Because these effects are dose dependent (30), responding patients with valproic acid levels above the therapeutic tolerance level had their dosage adjusted to lower their valproic therapeutic level. On the other hand, all of the resistant patients were gradually tapered off valproic acid therapy to initiate a trial on another line of AEDs. The protocol of the study is that patients whose seizures were not controlled at the end of the study were prescribed with other AEDs or switched to AEDs polytherapy without any further delay, which affected the compliance of patients to seizure control.
The efficacy of valproic acid (VPA) against epilepsy varies greatly between indi viduals,so the role of sodium channel genetic variation on VPA responsive is stil l being explored. As a result, it has been proposed that a shift in plasma level of VPA is not consistent among different responses at the third and sixth month of therapy, respectively. (Ebid et al) reported in their study that the therapeutic steady state did not guarantee clinical effectiveness of a drug (150).

4.4. Association of Clinical Parameters of the Epileptic Patients with the Response Rate After Three and Six months from Valproic Acid Treatment

The purpose of any antiepileptic therapy is reduce attack frequency, complete patients remission and improve quality of life. as a result, the clinical response of a patient may be the best evaluation criteria for efficacy, and in this

study population, the date, number, and type of seizures were documented on a daily record card, which was reviewed monthly at each clinic visit. The baseline seizure frequency was determined using the 3-month retrospective baseline. The efficacy endpoints included percent change in seizure frequency of any type per month from baseline over the treatment period, [ensure that valproic acid concentrations remain within the therapeutic window because therapeutic drug monitoring is recommended in epilepsy treatment (149)].

This current study design was in accordance with (Yu et al) in their study, which reported that efficacy re-evaluation procedures were conducted at baseline, 3rd month, and 6th month after enrolling for 6-month duration prospective study (151). Furthermore, (Nazish et al) shown in their 6-month prospective study that, while 6-month follow-up is short according to guidelines, most biochemical factors could be affected by AED during the 6th month and longer follow-up time affects patients' quality of life (152).

The seizures and epilepsy syndromes were classified according to the International League Against Epilepsy (ILAE) (153) In the present study the efficacy measures included the percent change in seizure frequency depending on detect numbers of seizure attacks of any type per month from baseline in the treatment period , this study conception was accordance with (Wang et al) in their study about response definition (154). Epileptic patients was divided into groups, first group had VPA- poor responsive was defined as a reduced percentage of seizure frequency (less than 50%) and second group was defined as reduced percentage of seizure frequency (more than or equal to 50%) was regarded as VPA-responsive (155,156).

Many studies defined drug-responsiveness as total seizure free, excluding all patients with seizures regardless of seizure frequency decrease. In clinical trials, however, a patient who had frequent seizures and experienced a significant decline in seizure frequency of 50% or more with the use of AEDs would be called a responder. As a result, not all definitions of intractability will be appropriate for all purposes (157), and different definition may be justified in different contexts. Definitions must be highly sensitive and specific. Although the creation of consensus criteria is certainly desired, intractable epilepsy is still described in a variety of ways (156).

As shown in the table (3-4), there was a significant reduction in the number of seizures per month in VPA responsive patients they had a reduction in attack frequency more than 50% when comparing baseline with the third and sixth months of valproic acid monotherpy.(p value 0.001, 0.001), and there was also a significant reduction in the number of seizures per month in poor responsive patients , but they had a reduction in attack frequency less than 50% when comparing baseline with the third and sixth months of valproic acid monotherpy (p value < 0.001 ,0.001 respectively),consistent with our findings, (Nazish HR et al) showed a significant reduction in the number of seizures in responsive patients at comparison baseline with 3rd month as well as baseline with 6th month of the VPA monotherapy. while there is no significant reduction in number of seizure in poor responders patients in their study (152).

4.5. The Frequency and Percentage of Detected Genotypes of SCN1A c.3184 (A>G) (rs2298771)) and UGT2B7 c.802 (C> T) (rs7439366) in the Iraqi Epileptic Patients

A mutation in the SCN1A gene, which codes for sodium channels, may result in altered receptor function and affect the efficacy of VPA, the SNP may change the structure or function of the sodium channel receptors, which may affect the VPA response (158). VPA is a type of histone deacetylase inhibitor that suppresses neuronal activity by inhibiting voltage-sensitive sodium channels (159). SNP rs2298771 (A > G)is a missense variation in SCN1A gene (158). This polymorphism causes the amino acid threonine to be substituted by alanine at a highly conserved position in the coding region of the SCN1A gene, potentially affecting the function of the inactivation gate in the cytosol that regulates sodium ion efflux and influx (160) .

As shown in the table (3-5), genotyping testing determined the frequency and percentage of SCN1A genotypes among the study's epileptic patients. In this study, the genotype (AA) of SCN1A was the most common genotype in epileptic Iraqi patients in terms of frequency and percentage (45,43.7%). corresponding to other genotypes (GG, GA) in the study. in Iranian Patients with Epilepsy reported SNP (p. A1067G; rs2298771) with allelic frequency as 0.7058/0.2942 (161) In North Endia population the genotype (AA) of SCN1A p. Thr1067Ala or c.3184 A/G (rs2298771) had frequency (42.9%) (160)

UGT2B7 is one of the UGT isozymes that play critical roles in the production of VPA glucuronides. VPA biotransformation is comprised of three major metabolic pathways: the uridine diphosphate glucuronosyltransferase

(UGT) enzyme pathway, the mitochondria -oxidation pathway, and the cytochrome P450 (CYP) pathway, which account for 50%, 40%, and 10%, respectively (162). The most common is glucuronidation conjugation, which is mediated mostly by UGT1A6 and UGT2B7. Because glucuronide metabolites represent for 50% of the VPA dose (163,164) .they play a crucial role in VPA metabolism. UGT2B7 glucuronidates many endogenous and exogenous substances, including steroid hormones, bile acid, and nonsteroidal anti-inflammatory medications (NSAIDs) (165)**.** This gene shows multiple polymorphisms in the proximal promoter region (166) UGT2B7 is a 16-kb gene on chromosome 4q13 that is composed up of six exons: the first two encode the substrate binding domain, while the final four encode the uridine 5'-diphosphate-glucuronic acid binding domain (127). UGT enzymes are important metabolic proteins that inhibit the accumulation of potentially toxic lipophilic substances and accelerate their removal via more hydrophilic biliary and renal systems. (167) .

Several polymorphisms have been discovered in the UGT2B7 gene. The C to T transversion at nucleotide c.802 results in a change in amino acid sequence, His268Try. This variant is known as UGT2B7*2, found in exon 2 of UGT2B7 (168).

rs7439366 (C > T, UGT2B7) is a missense variant of UGT2B7 that changes the amino acid from Tyr to His. The mutant genotype (TT) was the most common genotype in epileptic Iraqi patients with frequency and percentage (60,58.2%), while CC is a minor genotype with frequency and percentage (18,17.5%). Although the T and C alleles are equally abundant in northern and western European groups (MAF = 0.50, data from the 1000 Genomes database), and the frequency of the UGT2B7allele in Caucasians and

Asians is 48.9 and 26.8 %, respectively. (169) The C802T polymorphism is a frequent missense (MAF = 0.3349) ,the C allele is more common in Han Chinese (MAF = 0.722) and Japanese populations (MAF = 0.682) (170).

4.6. Association of Genotype SCN1A c.3184 A>G (rs2298771) Polymorphism in Epileptic Patients with Clinical Parameter and Response Rate After Three and Six Months of Valproic Acid Treatment

After genotypic analysis done in each patient the statistical analysis shows that there was a significant decrease in mean of attacks in epileptic patients with AA genotype these parameter were lower with rate ≥50 % from baseline as compared to GG and AG allele in three and six months which means that the presence of G allele of rs2298771 may cause a decrease in response to VPA that related to SCN1A, whereas AA carriers have a good response to valproic acid as compared with the other two as in the table (3-7).

Table (3-8) shows that there was a significant difference in number and percentage of three genotypes homozygous wild AA homozygous mutant GG and heterozygous AG of epileptic patients between the two difference responsive groups, so as AA genotyping had higher number and percentage in responsive group who had response rate ≥ 50% than AG and GG genotypes after three and six months (p value<0.001 ,<0.001 respectively), Moreover table (3-9) illustrate the epileptic patients' response to valproic acid therapy according to logistic regression analysis and by using the AA allele as reference there was a significant difference in response to valproic acid between the GG and AA and between AG and GG alleles in which the GG and AG have a lower

response extent as compared to AA allele after three and six months of the therapy.

So that A allele carriers had a better response to valproic acid than G allele carriers, indicating the presence of the mutant G allele of rs2298771 may be increased susceptibility to VPA resistance among Iraqi epileptic patients .

This study's findings confirmed the existence of a strong link between the G mutant allele of the SCN1A polymorphism and pharmacoresistant epilepsy. SCN1A encodes the alpha subunit of the type 1 voltage gated sodium channel, which regulates neuronal action potential initiation and propagation. (171,172). SCN1A is the most frequently mutated gene implicated in epileptogenesis (173), SCN1A (rs2298771) c.3184 A>G/p.Thr1067Ala is a missense protein coding polymorphism that results in a p.Thr1067Ala substitution, which may affect the structure-function relationships of the channel, leading to misfiring of brain neurons and explaining its association with epilepsy (160) . Moreover, impaired channel function as a drug target may affect its sensitivity to VPA, altering efficacy and patient response (174).

Many studies have explored the association between SCN1A gene polymorphis m and AED response, but few studies have focused on VPA response, and the re sults have been inconsistent (96).many investigations found that genetic variatio n has a significant relationship with AED response (175,176).

At present, many studies indicated that genetic polymorphism had a significant correlation with responsiveness of AEDs ,but a few studies found that mutant genotype or allele did not improve or reduce efficacy when using mono- or combination antiepileptic medications (177).

Consistent with our results, Li et al. demonstrated in a meta-analysis that the G allele and GG genotype of SCN1A rs2298771 increase the risk of AED resistance in the general population with epilepsy and in Asians with epilepsy and the AA genotype of SCN1A rs2298771 indicates sensitivity to AEDs in Caucasians (South Asians) (178).

However, additional studies showed an association between the SCN1A rs2298771 polymorphism and AED resistance. for example (Abo et al), found that the AG genotype and G allele frequency of SCN1A rs2298771 were significantly higher in AED-resistant patients than in drug-responders (179) also In a study to explore the effect of SCN1A rs2298771 genetic polymorphism on AED efficacy in Chinese patients with partial epilepsy, (Zhou et al) found a significantly lower level of drug efficacy among carriers of AG and GG genotypes compared to carriers of AA genotype, implying that the G allele may confer risk to drug resistant epilepsy (180).

A recent study in Egyptian epileptic children indicated that this polymorphism may have a role in an elevated risk of epilepsy and the development of drug resistance. There was a significantly higher frequency of the AG genotype and G allele of the SCN1A polymorphism in drug resistant individuals compared to drug responders (179) . Moreover a significant association was found between rs2298771 and CBZ resistance in patients of Asian origin with epilepsy. the results indicate that patients of Asian had relation between the epilepsy incidence and SCN1A polymorphism rs2298771, especially with the GG genotype, may be at risk of CBZ resistance (181)

In contrast, a study in Solvenia reported that carriers of at least one polymorphism SCN1A c.3184A > G /p.Thr1067Ala G variant had a decreased

risk of epilepsy and were more likely to achieve remission , despite the fact that it did not approach statistical significance, it suggests that this variant may play a protective role and may be connected with treatment efficacy (43). Other study in Chinese epileptic patients with focal seizures found that c.3184A > G/p.Thr1067Ala was significantly associated with AED monotherapy efficacy (174)

While several studies have failed to find any effects of this polymorphism on the drug-resistant phenotype in the patients with epilepsy(146,160,180). For example, (Lakhan et al) found that the AG genotype of SCN1A c.3184 A/G polymorphism was significantly associated with epilepsy but showed no significant association with drug resistance in north Indians (160). The variations between the studies could be attributed to a variety of factors, including genetic differences, sample size, cohort age distribution, environmental factors (176).

In Conclusion, the findings demonstrate that variant genotypes of the (AG and GG) carriers of SCN1A (rs2298771) c.3184 A>G/p.Thr1067Ala are significantly associated with poor response to VPA monotherapy, even when plasma levels are within the therapeutic range, pointing to possible genetic polymorphism and pharmacodynamic interactions.

4.7. Association of UGT2B7 c.802C>T (7439366) Genetic Polymorphism with Valproic Acid Concentration and Response Rate After Three and Six Months of Treatment

VPA showed substantial interindividual variability in steady-state serum levels, which influences its therapeutic effects (182). Furthermore, following clinical studies, VPA was found to have a variety of adverse effects especially liver toxicity (183) .As a result, dosage optimization was critical in epilepsy treatment and genetic polymorphisms that may have an impact on the pharmacokinetics and pharmacodynamics of VPA could assist in the improvement of individualized therapy (116)

The dosing schedule was maintained for at least 2 weeks (>5 half-lives) to establish a steady-state condition and due to considerable inter-individual variations and to avoid the error from weights and dosages, the VPA serum concentration at steady-state were adjusted by body weight and dosage of the each patient and represented as adjusted concentration of VPA (AC VPA, µg/mL per mg/kg) (62).

In the present study after genotypic analysis done in each patient the statistical analysis shows that there was a significant difference in the mean ±SD of the adjusted concentration of VPA (AC of VPA) which was much lower in patients with a T allele at UGT2B7 (7439366) C802T than in those with a CC genotype as in the table (3-10). These findings suggest that C802T polymorphisms influence UGT2B7 enzyme activity and VPA metabolism and may be an important determinant of individual variability in VPA pharmacokinetics.

The current study confirmed with (Sun et al), who revealed that the UGT2B7 C802T SNP was significantly associated with VPA CS, and that the incidence was much lower in patients with one or more T allele carriers than in patients with the CC genotype, these findings suggested that UGT2B7 plays an important role in VPA metabolism; gene mutations can cause both transcriptional and functional alterations in this enzyme, which may increase VPA metabolism, in individuals with the T allele at C802T, the VPA dose may need to be increased to achieve the therapeutic range of 50–100 μg/mL (184).

The current study agreed with (Du Z et al) study which found that plasma concentrations of VPA are higher in patients with the genotype UGT2B7 802 CC than in those with the genotypes CT or TT, indicating a high metabolism in patients with the T genotype (185).

On the other hand, several others investigations, found that UGT2B7 C802T had no effect on plasma VPA levels or VPA metabolism (111,113,179) .Various research on other UGT enzymes have found that gene polymorphisms alter enzyme activity and produce a reduction in VPA concentration in patients who carry mutant alleles. Two missense variants in exon that result in enhanced enzyme activity and may impair VPA concentration are rs6759892 (T > G, UGT1A6, MAF = 0.260) and rs1105879 (A > C, UGT1A6, MAF = 0.262). As a result of these two variances, carriers may require a greater VPA dose. (110,187)

This study made further analysis on efficacy association of UGT2B7 c.802 C>T polymophism on efficacy between responders and non-responders groups the results have shown that there are no significant different frequency and percentage in the this SNP between responders and non-responders groups

as in the (table 3-11) and (table 3-12). So The results show that C802T polymorphisms exert influences on the serum concentration of VPA, while had no significant influence on VPA efficacy

While many studies have illustrated that lower plasma VPA concentrations were associated with pharmacoresistant patients compared with responsive patients which could be the main factor of pharmacoresistant epilepsy such as a study by (Du Z et al) reported that further evaluation on efficacy indicate the C802T polymorphism could affect the efficacy of VPA. and the results cannot completely clarify the regulatory mechanism, there are genes interactions that may play specific roles in the metabolism of this drug. Furthermore, changes in patient constitution, nutrition, and behavior may influence drug metabolism and efficacy. More research is required on this issue. (188).

The results of studies were various due to number of factors firstly, sample size is a critical factor in association analysis, which may result in poor power (59). furthermore, the fact the polymorphisms in UGT2B7 have shown to alter the transcription level or the capability of metabolic enzymes, the function of these metabolic enzymes could be eliminated by the overexpression of others (128).

The findings of this study could help to improve therapy for epileptic patients. UGT2B7 C802T has previously been identified as an important determinant of variability in the pharmacokinetics of VPA. Furthermore, it is suggested that there are significant associations of this SNP that may play specific role in metabolism and VPA concentration with no significant affecting on therapeutic efficacy, implying that more research on this topic is required.

4.8. Association of UGT2B7 (C> T) (rs7439366) Polymorphism with the Serum Liver Enzymes Level before and after VPA Treatment and Susceptibility to Hepatotoxicity as Valproic Acid Adverse Effect

Hepatotoxicity, an idiosyncratic and fatal adverse drug reactions linked with VPA treatment, occurs at an incidence of over 1/40,000 in adults, 1/5000 in children, and 1/500 in high-risk populations, these occurrences have typically happened during the first six months of treatment. non-specific symptoms such as malaise, weakness, lethargy, facial edema, anorexia, and vomiting may follow serious hepatotoxicity (189). Valproic acid has a narrow therapeutic range and substantial inter-individual variability in pharmacokinetics and pharmacodynamics; thus, its plasma level (50–100 μg/mL) must be closely monitored during course of treatment to avoid resistance as well as toxicity (182). One of the most serious adverse drug reactions is hepatotoxicity, which limits its clinical use. The National Institutes of Health (NIH) has issued a caution to patients regarding the potential of serious or fatal liver injury from VPA applications (190).

Valproic acid commonly cause a symptomatic increase in the liver enzymes and serum bilirubin, although the exact mechanism of VPA-induced hepatotoxicity is unclear, many studies have suggested that the synthesis and accumulation of reactive metabolites in hepatocytes plays an important role in this pathogenesis (191,192). VPA hepatotoxicity has been related to the generation of VPA reactive metabolites, suppression of fatty acid -oxidation, excessive oxidative stress, and genetic variations of several enzymes, including CPS1, POLG, GSTs, SOD2, **UGTs**, and CYPs genes. the impact of inheritable

polymorphisms on human UGT-encoding genes has been extensively studied and has been demonstrated to account for a fraction of the reported phenotypic variation in metabolism and excretion (167). Many repeated polymorphism reduces expression levels by modifying transcription initiation and it also reduces glucuronidation of bilirubin and other substrates by approximately 70% (193,194).

VPA is eliminated through substantial biotransformation via several pathways, including mitochondrion-mediated β-oxidation, cytochrome P-450 (CYP450)-catalyzed oxidation and glucuronidation catalysis. The primary hepatotoxic metabolites of VPA are 2-propyl-4-pentenoic acid (4-ene-VPA, a result of CYP450-catalyzed oxidation) and 2-propyl-2,4-pentadienoic acid (2,4-diene-VPA,the β-oxidation metabolite) (195), Furthermore, another unsaturated metabolite derived from mitochondrial β-oxidation, 2-propyl-2-pentenoic acid (2-ene-VPA), could be transformed to 2,4-diene-VPA in vivo (196)**.**

. Children's UGT-mediated metabolism is reduced because they have lower activity of UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15 than adults, this could lead to higher liver damage in young children. moreover, age-dependent changes in mitochondrial function have been discovered in children undergoing VPA treatment, indicating that developmental alterations in mitochondrial function are occurring. the incidence of fatal hepatotoxicity decreases considerably in progressively older patient groups (88,197)

The current study investigated liver function test (LFTs) liver function tests including alanine aminotransferase(ALT), aspartate aminotransferase (AST), and serum bilirubin at baseline and then after the first 6 months of

treatment of valproate monotherapy to determine effect of the UGT2B7 c.802 polymorphism in serum liver enzyme levels and increased risk factors for VPA-induced hepatotoxicity

Study design was in accordance with previous study that reported the patients whose baseline and follow-up liver function tests and serum bilirubin test that were measured before and in the treatment period after first six months from enrolled to evaluated hepatic enzyme and serum bilirubin level (198)

alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum bilirubin aminotransferases are conventionally markers been associated with hepatocellular injury (199) the release of intracellular enzymes such as AST, ALT is a marked indicator for hepatocyte injury ,serum AST, ALT are present in the cytoplasm of most cells and their release into the circulation infer cellular damage (200)

As shown in table (3-13), the present study findings revealed a significant increase in ALT, AST, and bilirubin. (within normal range) among homozygous CC genotype of UGT2B7 (rs7439366) (C>T) genetic polymorphism after valproic acid therapy (p value 0.001, 0.004, 0.001 respectively). , bilirubin levels increased significantly in CT genotype carriers (p value 0.004), but there was no significant association of serum liver enzyme ALT, AST among heterozygous CT carriers. Similarly, there is no significant correlation between ALT, AST, or bilirubin levels in the mutant homozygous TT genotype carriers.

This variation could be attributed to carriers with the CC genotype having higher mean VPA concentrations than CT,TT genotypes . It is possible

that high VPA serum levels are one of the factors that increase susceptibility to VPA hepatotoxicity by altering VPA metabolism pathway (201) implying a lower enzymatic activity that may down regulate the UGT pathways. Subsequent compensatory enhancement in CYPs may be observed, and 4-ene-VPA and 2,4-diene-VPA generation may be increased to some extent (202). therefore high risk of affected hepatocellular function which caused elevated liver enzyme AST, ALT, Bilirubin in CC genotype and Bilirubin in CT genotype, careful administration is especially necessary for patients who are UGT2B7 802 C allele carrier.

The current study agreed with (Zhao et al.) in their study that found that patients with higher adjusted serum valproic acid levels were more susceptible to hepatotoxicity, but disagreed with them in that T allele carriers of the UGT2B7 C802T gene polymorphism had higher adjusted VPA concentration , because the current study found that C allele carriers had higher AC of VPA than other alleles in Iraqi populations (202).

Another previous study corresponding with the current study hypothesis which was the generation of hepatotoxic metabolites by CYPs may be indirectly influenced, given that CYP450-mediated oxidation plays a minor role in VPA's metabolic pathway, it is unlikely that CYP450 has a significant impact on VPA's efficacy and adverse event profile. Although the UGT pathway is not a direct or primary pathway for the formation of hepatotoxin metabolites, UGT pathway may have an indirect effect on CYPs. There has been little research focused on the role of these UGT enzymes in the serum concentrations of hepatotoxic metabolites, this hypothesized was in agreement with the study in China cohort (202) .

Most previous investigations focused on the direct influence of CYPs on the generation of hepatotoxic metabolites (203),found that genetic polymorphisms of genes encoding CYP450 enzymes (CYP2C9, CYP2C19, CYP2A6, and CYP2B6) influence VPA and its metabolites in vivo because of the exceedingly complicated metabolic profiles (196,204) this may neglected the mutual interference among different metabolic pathways. The action of CYP450 enzymes accounts for only about 10% of VPA elimination, whereas glucuronidation accounts for roughly 50% of VPA metabolism and is thus the most critical factor determining the administered VPA dose (205).

In conclusion, this is the first study to examine the impact of UGT2B7 genetic polymorphisms to the elevation of primary liver enzymes in epileptic Iraqi patients treated with valproic acid for six months had normal liver function prior to therapy. The findings of this study suggest that determining UGT2B7 C802T polymorphisms, serum VPA concentration, and serum AST, ALT, and Total bilirubin concentrations in VPA therapy may be clinically useful as indices for the prediction and prevention of VPA-induced hepatotoxicity and can help decrease VPA adverse drug reactions (ADRs) in high-risk patients. This shows that people with the CC genotype of UGT2B7 c.802C>T should be monitored more closely for the possibility of hepatotoxicity.

4.9. Conclusion

From the results of this study, we can conclude that**:**

1. The application of molecular biology to study the polymorphism of the site of action and metabolism enzyme of valproic acid has made a significant progress in understanding the valproic acid efficacy in epilepsy disease.
2. The homozygous wild (AA), homozygous mutant genotype (GG), and heterozygous genotype (AG) of SCN1A rs2298771 were detected in different frequencies by using AS-PCR in Iraqi epileptic patients. The AA genotype of SCN1A (A>G) (rs2298771) genetic polymorphism is more predominant than other genotypes which were GG and AG.
3. SCN1A polymorphism (rs2298771) causes a decrease in response to valproic acid treatment in mutant alleles (GG) and heterozygous (AG) as compared to reference alleles (AA).
4. The homozygous wild (CC), homozygous mutant genotype (TT), and heterozygous genotype (CT) of UGT2B7 c.802 C>T rs7439366 were detected in different frequencies by using ARMS-PCR in Iraqi epileptic patients. The TT(mutant) genetic polymorphism is more predominant than other genotypes which were CC and CT.
5. UGT2B7 c.802 C>T (rs7439366) polymorphism causes a decrease in valproic acid adjusted concentration in mutant alleles (TT) and heterozygous (CT) as compared to wild alleles (CC).
6. People with the CC genotype of UGT2B7 c.802 C>T (rs7439366) should be monitored liver function test more closely for the possibility of hepatotoxicity after six months treatment.

4.10. Recommendations and Future work

1. Preferably studies with larger sample size patients and control are needed to clarify the influence of SCN1A polymorphism in the genetic susceptibility of idiopathic epilepsy, advanced molecular studies such as gene sequencing can be more effective in the detection of gene.
2. Investigate another SNPs of SCN1A gene and UGT2B7 gene and examine their effects on the response of valproic acid.
3. Studies with larger sample size are needed to clarify the role of UGT2B7 c.802 C>T (rs7439366) variants on valproic acid response.
4. Investigate another genes polymorphism and their roles in hepatotoxicity after valproic acid treatment.

In a practice situation, Therapeutic drug monitoring (TDM) and regular liver biochemical monitoring throughout VPA therapy, as well as genotype screening for specific patients before to VPA administration, should improve the safety and efficacy profile of this antiepileptic drug.

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Appendices

Questionnaire of Epileptic Patients

- **Name:** **Gender:**
- **Age:** **Weight:**
- **Onset of illness:**
- **Hereditary disease:**
- **Family history:**
- **Neurological disease:**
- **Etiology of epilepsy:**
- **Type of fit:**

  Focal:

  Generalized:
- **Frequency of fit in three months ago:**

  Control:

  Not control:
- **Type of Antiepileptic treatment :**

  No AED:

  With AED : **regular:** **irregular:**
- **History of medication, alcohol, drugs abuse:**

كارت المريض

اسم المريض: **الجنس:** **العمر:**

تاريخ البدء بالعلاج: **الجرعة :**

الشهر الاول من العلاج							
	السبت	الاحد	الاثنين	الثلاثاء	الاربعاء	الخميس	الجمعة
الاسبوع الاول							
الاسبوع الثاني							
الاسبوع الثالث							
الاسبوع الرابع							
الاسبوع الخامس							
المجموع							
تسجل الشمرة الواحدة على شكل ا في حال حدثت اكثر من شمرة خلال اليوم تكتب اا ,ااا,... وهكذا **ملاحظات**							

الشهر الثاني من العلاج							
	السبت	الاحد	الاثنين	الثلاثاء	الاربعاء	الخميس	الجمعة
الاسبوع الاول							
الاسبوع الثاني							
الاسبوع الثالث							
الاسبوع الرابع							
الاسبوع الخامس							
المجموع							
تسجل الشمرة الواحدة على شكل ا في حال حدثت اكثر من شمرة خلال اليوم تكتب اا ,ااا,... وهكذا **ملاحظات**							

الشهر الثالث من العلاج							
	السبت	الاحد	الاثنين	الثلاثاء	الاربعاء	الخميس	الجمعة
الاسبوع الاول							
الاسبوع الثاني							
الاسبوع الثالث							
الاسبوع الرابع							
الاسبوع الخامس							
المجموع							
تسجل الشمرة الواحدة على شكل ا في حال حدثت اكثر من شمرة خلال اليوم تكتب اا ,ااا,... وهكذا **ملاحظات**							

الشهر الرابع من العلاج							
	السبت	الاحد	الاثنين	الثلاثاء	الاربعاء	الخميس	الجمعة
الاسبوع الاول							
الاسبوع الثاني							
الاسبوع الثالث							
الاسبوع الرابع							

الاسبوع الخامس							
المجموع							
تسجل الشمرة الواحدة على شكل ا في حال حدثت اكثر من شمرة خلال اليوم تكتب ا ,اا,... وهكذا **ملاحظات**							
الشهر الخامس من العلاج							
	السبت	الاحد	الاثنين	الثلاثاء	الاربعاء	الخميس	الجمعة
الاسبوع الاول							
الاسبوع الثاني							
الاسبوع الثالث							
الاسبوع الرابع							
الاسبوع الخامس							
المجموع							
تسجل الشمرة الواحدة على شكل ا في حال حدثت اكثر من شمرة خلال اليوم تكتب ا ,اا,... وهكذا **ملاحظات**							
الشهر السادس من العلاج							
	السبت	الاحد	الاثنين	الثلاثاء	الاربعاء	الخميس	الجمعة
الاسبوع الاول							
الاسبوع الثاني							
الاسبوع الثالث							
الاسبوع الرابع							
الاسبوع الخامس							
المجموع							
تسجل الشمرة الواحدة على شكل ا في حال حدثت اكثر من شمرة خلال اليوم تكتب ا ,اا,... وهكذا **ملاحظات**							

الأشكال (rs7439366) UGT2B7 C802T على استقلاب VPA وتركيزه في مصل الدم و تكون عاملاً محددًا مهمًا للتباين الفردي في الحرائك الدوائية لـ VPA في النمط الوراثي المتحولT أليل. علاوة على ذلك ، يُظهر تحديد الأشكال المتعددة الأشكال UGT2B7 C802T أنه يجب مراقبة الأشخاص الذين لديهم النمط الوراثي CC لـ UGT2B7 (rs7439366) C802T عن كثب لاحتمالية السمية الكبدية.

على مستوى إنزيمات الكبد في الدم لتقدير عوامل الخطر الوراثية للتسمم الكبدي الناجم عن VPA في السكان المصابين بالصرع في محافظة كربلاء.

المرضى والوسائل :

هذه الدراسة هي دراسة مستقبلية أجريت في استشارية الأمراض العصبية في مدينة الإمام الحسين الطبية / كربلاء - العراق وعيادة خارجية تم فيها اختيار المرضى الذين تم تشخيصهم حديثًا وغيرهم ممن يعانون من فشل سابق في العلاج من أكتوبر 2020 إلى نوفمبر 2021 أثناء طلب الرعاية الطبية . تم اختيارهم اعتمادًا على تاريخهم الطبي والمظاهر السريرية للصرع والاعتماد على معايير تشخيص الرابطة الدولية لمكافحة الصرع (ILAE). أجريت هذه الدراسة على مائة وثلاثة عشر مريض مصابين بالصرع وقد بدأوا باستخدام حمض الفالبرويك الأحادي 10-15 مجم / كجم منذ بداية الدراسة (لمدة 6 أشهر على الأقل) تم جمع البيانات السريرية شهريًا لكل مريض احتفظ بسجل للتاريخ والعدد ومدة هجمه نوبات الصرع العصبية على بطاقة السجل اليومية ، تمت عمليات إعادة التقييم في الشهر الثالث والسادس بعد التسجيل. تم استخدام خط الأساس بأثر رجعي لمدة 3 أشهر لتحديد وتيرة الضبط الأساسي. تم جمع عينات الدم من كل مريض وافق على الاختبار الجيني وقياس تركيز حمض الفالبرويك في الدم في حالة مستقرة بعد ثلاثة وستة أشهر واختبار إنزيمات الكبد (AST)، (ALT)، (Total bilirubin) قبل وبعد ستة أشهر من العلاج.

النتائج:

في هذه الدراسة كان هناك ارتباط ذو دلالة إحصائية بين تعدد الأشكال الجيني SCN1A rs2298771 c.3184 A / G مع الفروق في العلامات السريرية واستجابة VPA وكان هناك ارتباط ذو دلالة إحصائية بين UGT2B7 (rs7439366) c.802 C/T تعدد الأشكال مع الفروق في تركيز VPA في المصل , والبعض من إنزيمات الكبد كتأثير جانبي ، في حين أن هذا SNP ليس له ارتباط معنوي مع استجابة حمض الفالبرويك بين مرضى الصرع.

الاستنتاج:

اظهرت النتائج ان تعدد الأشكال الجيني لـ SCN1A rs2298771 c.3184 A / G مرتبط بزيادة حدوث المقاومة لدواء حمض الفالبرويك خلال مدة العلاج في النمط الوراثي المتحول G اليل. و قد تؤثر تعدد

الخلاصة

خلفيه الدراسة :

يتم استخدام حجب قناة الصوديوم بحمض الفالبرويك على نطاق واسع في مرضى الصرع البالغين والأطفال. قنوات الصوديوم الرئيسية في الجهاز العصبي المركزي يتم ترميزها بواسطة جينSCN1A في أغلب الأحيان ترتبط طفرات SCN1A بمجموعة متنوعة من شدة الصرع الوراثي و قد تكون عوامل رئيسية للفروق المظهرية الفردية للاستجابة لمضادات الصرع تعتبر مقاومة الأدوية معقدة ، وبالتالي فإن فعالية حمض الفالبرويك (VPA) تختلف بشكل كبير في العلاج السريري لمرضى الصرع التي تم ربطها بـتعدد الأشكال في الجينات المشاركة في موقع عمل حمض الفالبرويك مما أدى إلى الاعتقاد بأن النمط الجيني SCN1A قد يلعب دورًا في استجابة حمض الفالبرويك .العامل الثاني وهو أن تركيز الحالة المستقرة لـ VPA في الموقع المستهدف يتأثر بالاختلافات الجينية في امتصاصه ونقله واستقلابه. UGT2B7 هو إنزيم رئيسي في عملية التمثيل الغذائي لـ VPA . الطفرات الوظيفية لـ UGT2B7 وقد تكون أحد المتنبئين للتغيرات الفردية في الحرائك الدوائية لـ VPA ، ويجب تعديل جرعة VPA لدى الأفراد إلى النطاق العلاجي لضمان الإنجاز ولتجنب التفاعلات الدوائية الضارة لـ VPA مثل السمية الكبدية في المرضى المعرضين لمخاطر عالية. في هذه الدراسة تم التنميط الجيني لاثنين من أشكال النوكليوتيدات المفردة (SNPs) في اثنين من الجينات المحتملة المرتبطة بموقع عمل المستقبل وإنزيم استقلاب الدواء.

أهداف الدراسة:

صممت الدراسة الحالية للتحقق من وجود وتوزيع SCN1A (rs2298771) c.3184 A / G و UGT2B7 (rs7439366) C802T تعدد الأشكال الوراثي بين مرضى الصرع وكذلك لدراسة تأثير تعدد الأشكال الجيني في جينات SCN1A (rs2298771) c.3184 A / G و UGT2B7 (rs7439366) C802T على استجابة VPA عبر التباين المحدد في ديناميكيات الدواء و الحرائك الدوائية لـ VPA. علاوة على ذلك ، بحثت هذه الدراسة في تأثير تعدد الأشكال الجيني ل UGT2B7 C802T

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الصيدلة

دور تعدد الأشكال الجينية SCN1A و UGT2B7 في الاستجابة لحمض الفالبرويك بين مرضى الصرع العراقيين

رسالة مقدمه الى

كليه الصيدلة جامعه كربلاء كجزء من متطلبات نيل شهادة درجه الماجستير في الأدوية والسموم

من قبل

ايات عبد الخالق جواد كاظم

بكلوريوس صيدلة/ الجامعة المستنصرية

بإشراف

ا.م.د. احمد حقي إسماعيل

دكتوراه أدوية وعلاجات

ا.م.د. الاء عبدالحسن حمدان

دكتوراه تقنيات احيائيه

2022 ميلادي

1443 هجري