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College of Applied Medical Sciences

Comparison the Level of Biomarker Tetranectin, Protein S100B and High-Mobility Group Box-1 Protein in Patients with Primary and Secondary Epilepsy

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Dedication

I dedicate the fruits of my efforts to the one who gave me life, hope, and growth based on a passion for knowledge and learning, my dear father.

To the light of my eyes and the beat of my heart, to the one who surrounded me with her prayers and love until I became who I am, to my first inspiration and my source of strength, without you, I wouldn't have made it, my lovely mother.

To whom God gave me their presence in my life my sisters and brother.

To the pure and gentle heart that still guides me, who shows me how to give, how to love, how to sacrifice to my dear husband.

To my blessed grace that brought light and determination into my life, to my beloved son (Mustafa)

I thank everyone whose effort has put a stone in building up this work.

To everyone who supported me even with a smile.

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Summary

Epilepsy is a neurological disorder, characterized by the persistent propensity to have seizures. It is categorized based on the major cause of the disease to primary epilepsy which includes genetics cause, and secondary epilepsy which includes acquired causes of this disease.

This study aimed to differentiate between patients with epilepsy and healthy people as well as to differentiate wither patients having seizure due to genetic predisposition (primary epilepsy) or due to acquired causes (secondary epilepsy) by measuring the levels of Tetranectine, Protein S100B and high-mobility group box 1 (HMGB1) biomarkers which can also be used to predict the possibility of having epilepsy disease.

The study was carried out at laboratories of Al-kafeel hospital and the EEG unit of Al-Hussein Teaching Hospital in Karbala, from November 2024 to April 2025. There were 90 participants in the study, their ages range from 15 to 60. Thirty of them are healthy people (control group) and thirty with primary epilepsy and thirty with secondary epilepsy (patients) were included.

According to results of this study there was a significant difference observed in the levels of Tetranectine between the control group and both primary and secondary patient groups as it increased significantly in the secondary group.

There were statistically significant differences in the levels of protein S100B and HMGB1 biomarkers between the control group and patients with primary and secondary epilepsy with higher level of these biomarkers in the secondary group.

The study shows that the tetranectine levels increase gradually in younger patients from control groups to primary group to secondary group, but in patients aged 30 or older, it increases sharply until it reaches the highest level in the secondary

group, this indicates that the older individuals may experience more severe disease response, also the Protein S100B in both age groups show a significant rise in levels from controls to secondary patients, indicating a highly significant difference between the groups. Similarly, HMGB1 levels increase in both groups, with slightly higher levels in younger secondary patients. All changes are statistically significant (p-values < 0.01).

The study's findings showed significant difference in the level of creatinine ($P<0.01$)-slightly lower in primary epilepsy compared to controls and secondary as well as a significant decrease in the levels of Ca++ and HBA1C in patients for both groups especially in secondary patient group compared with control group.

There were observed correlations among the research parameters. For example, Tetranectine exhibited a positive relationship with HMGB1 and Protein S100B. Furthermore, a significant negative correlation was found between HMGB1 and calcium levels. Additionally, HMGB1 was positively correlated with Protein S100B, while also showing negative correlations with calcium and HBA1C. A strong relationship was identified between seizure duration and age, as well as with BMI. Tetranectine, S100B, and HMGB1 also displayed positive but non-significant associations with seizure duration.

The study also focused on the prediction possibility of epilepsy disease wither it has primary or secondary cause and the results show that the best diagnostic performance was shown by Protein S100B, which had demonstrating a high capacity to accurately detect positive cases. HMGB1 also showed high performance, and a more balanced diagnostic profile. Tetranectine, while having the lowest AUC, still demonstrated acceptable sensitivity and specificity.

According to the results mentioned above, these biomarkers: Tetranectin, Protein S100B, and HMGB1 could be used to differentiate between patients with epilepsy, either primary or secondary and to predication the possibility of having this disease.

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List of Abbreviations

Abbreviations	Descriptions/Full forms
AKD	acute kidney damage
AKI	Acute Kidney injury
ALS	Amyotrophic lateral sclerosis
AED	antiepileptic drugs
AUC	area under the curve
Aβ	amyloid-beta
BBB	Blood Brain Barrier
BUN	Blood Urea Nitrogen
BMI	Body Mass Index
Ca++	Calcium
CNS	Central nervous system
CSF	Cerebrospinal fluid
CKD	Chronic Kidney Disease
C	Control area
Co2	carbon dioxide
DEE	Developmental and epileptic encephalopathies
DER	drug-resistant epilepsy
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
eGFR	estimated glomerular filtration rate

GABA	Gamma-aminobutyric acid
HBA1C	Hemoglobin A1c
HRP	haptoglobin-related protein
HF	Heart failure
HMGB1	High-mobility group box 1
iNOS	inducible nitric oxide synthase
IL-1β	interleukin-1β
MAPK	mitogen-activated protein kinases
MCD	Malformations of cortical development
MTLE	Medial temporal lobe epilepsy
NH3	ammonia
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NREM	non-Rapid Eye Movement
CPC	O-Cresol Phtalein Complexone
OD	Optical density
K+	Potassium
ROS	Reactive oxygen species
ROC	Receiver Operating Characteristic Curve
RAGE	Receptor for Advanced Glycation Endproducts
Na+	Sodium
S100B	Protin s100B
SD	Standard Deviation
SMD	standardized mean difference
SPSS	Statistical Package for Social Sciences
TLE	Temporal lobe epilepsy
T	Test area
TLR-2	Toll-Like Receptor-2
TLR-4	Toll-Like Receptor-4
TBI	Traumatic brain injury
VGCCs	voltage-gated calcium channels
WHO	World Health Organization

Chapter One

Introduction

1.1 Introduction

The defining characteristic of epilepsy, a persistent neurodegenerative disorder, is the ongoing susceptibility to seizures. This condition is also linked to a diverse range of neurobiological, cognitive, psychosocial, and social comorbidities resulting from the repeated occurrence of seizure activity (Beghi, 2020). To diagnose epilepsy, one of the following criteria must be satisfied: either the occurrence of a single unprovoked seizure with a significant likelihood of subsequent seizures (analogous to the recurrence risk following two or more unprovoked seizures), or the experience of two or more unprovoked seizures that are spaced more than 24 hours apart (Fisher, *et al.* (2014).

Generally speaking, seizures fall into one of two main categories: focal or generalized. A particular area of the brain is the site of focal seizures, which can happen with or without decreased awareness. In contrast, generalized seizures involve both cerebral hemispheres from the onset and usually result in a loss of consciousness. Accurate identification and classification of seizure types are essential for establishing an appropriate diagnosis and guiding effective treatment strategies (Fisher *et al*., 2017).

The physiology of epilepsy involves complex interactions among neuronal excitability, synaptic transmission, and inhibitory mechanisms in the brain. Electrical signals are used by neurons to communicate, and when excitatory neurotransmitters such as glutamate predominate, this can result in greater firing of the neurons. (Lothman and Melendez. (2002)). Conversely, inhibitory neurotransmitters such as gamma-aminobutyric acid (GABA) help to dampen excessive neuronal activity (Huberfeld and Kokaia. (2012)). A disturbance in this equilibrium in epilepsy may cause neuronal circuits to become

hyperexcited, which can result in the occurrence of spontaneous seizures. (Engel. (2013). Furthermore, aberrant neural connections or gliosis are examples of structural abnormalities in the brain that might add to the pathophysiology of epilepsy. (Moshé, *et al.* (2015).

There are two types of epilepsy; Primary epilepsy is the epilepsy that occurs for a genetic reason, and secondary epilepsy is the acquired epilepsy that occurs for several reasons on the basis of which the parameters were chosen in this study.

Primary epilepsy may occur as a result of genetic alterations. Recurrent seizures can result from inherited genetic abnormalities that impair brain function, the primary cause of genetic epilepsy. These mutations can affect the release of neurotransmitters, ion channels, synaptic plasticity, and other biological mechanisms. (Poduri and Condro. (2018).

Acquired epilepsy (also known as secondary epilepsy) refers to seizure disorders that develop as a consequence of identifiable brain insults or underlying conditions, such as brain tumors, central nervous system infections, stroke, or traumatic brain damage (Fisher *et al.,* 2014).

Recent attention has been directed toward tetranectin, a matricellular protein involved in neuronal development and functional regulation, due to its potential role in epilepsy diagnosis and pathophysiology (Nakagawa et al., 2020). A study suggests that tetranectin may serve as a biomarker for epileptic activity and may reflect pathological processes occurring within the central nervous system. (Dahiya *et al.,* (2017).

The calcium-binding proteins S100B, mainly coming from astrocytes, have also been extensively seen to submit into neurological disorders (such as

epilepsy) (Fazzi, *et al.,* (2022). S100B has been found to be increased in the CSF and serum of epilepsy patients and decreased in the healthy subjects (He, *et al,.* 2024). As well as the diagnostic role, S100B may have active aetiologic effects in mediating seizures by modulating astrocyte functions, neurotransmitter release, and thereby leading to increased neural excitability (Moudgil *et al.,* 2023).

High-mobility group box 1(HMGB1) is a nuclear protein that has been implicated in the pathophysiology of epilepsy and has various cellular functions like inflammation and neural transmission. (Zhou*, et al.,* 2021). Elevated brain HMGB1 levels have been associated with neuro-inflammation and excitotoxicity, which can aggravate seizure activity or promote the development of comorbidities of epilepsy. (Maroso *et al.,* 2010)).

Hemoglobin A1c(HbA1c) is one of the key biomarkers for long term monitoring of glucose control in diabetics. (ElSayed*, et al.,* (2023). The HbA1c monitoring is useful for diagnosing patients with seizure in whom hyperglycemia or hypoglycemia is one of the causes of epileptic seizure (Hamaguchi, *et al.,* (2008)).

Two important chemicals that are frequently evaluated in blood and urine tests to evaluate kidney function and general metabolic health are urea and creatinine. (Kaur and Kaur, (2023).

In the neurological system, calcium is essential for signal transmission. It facilitates communication among nerve cells by aiding in the release of neurotransmitters at synapses. (Kandel, *et al.* (2013)).

1.2 Aims of study

- In the current work, we propose to compare primary and secondary epilepsy, in relationship with identifying and confirming the serum levels of selected biomarkers (Tetranectin, Protein S100B, High-Mobility Group Box 1 (HMGB1)), as the potential prognostic and diagnostic markers during epileptic patient follow-up. These biomarkers are explored for their capacity to identify the presence, severity and epileptogenic giant potential of brain tissue that is capable of giving rise, to spontaneous recurrent seizures.
- Besides biomarker profiling, the study is designed to test HbA1c, urea, creatinine and calcium measurements as factors to elucidate potential central metabolic loosening and co-morbidities related to epileptic diseases.
- A further objective is to examine the utility of these biomarkers in predicting the development and progression of epilepsy, particularly in at-risk individuals. The study also seeks to assess their role in monitoring disease course post-diagnosis and in stratifying patients for inclusion in clinical trials. This approach may help to optimize resource allocation, reduce the cost of epilepsy-related clinical trials, and enhance their efficacy by selectively enrolling individuals with a high risk of epileptogenesis.

Chapter Two

Literatures Revie

2.1 physiology of the brain

The brain is an incredibly sophisticated system of billions of neurons that communicate through complex chemical and electrical signals. The neuron, the basic functional unit in the nervous system, sends electrical impulses to adjacent neurons, though synapses and down its axon, using numerous neurotransmitters as mediators of this communication (Bear *et al.,* 2015). Different brain regions are dedicated to different operations; for example, the brainstem regulates life-sustaining autonomic functions (e.g., breathing and heart rate), and the cerebral cortex coordinates higher-order activities such as perception, thought, and voluntary movement (Kandel, *et al.,* 2013).

The blood-brain barrier (BBB) serves as an essential selective permeability interface allowing essential nutrients to enter the brain, while protecting the neural tissue from the deleterious compounds present in blood (Pardridge, 2012). Additionally, learning, memory consolidation and recovery from injury are facilitated by the brain's extraordinary ability to adapt (known as neuroplasticity), which allows neural circuits to rewire in response to experience or injury. (Johnson, 2023).

2.2 Neurons and Synapses

Neurons are the fundamental structural and functional units of the nervous system. A cell body (or soma) and dendrites, which receive incoming signals comprise each neuron in addition to a single axon that transmits action potentials (electrical impulses) to other neurons or to effector organs/tissues (Koch, 2004). Neurotransmitters are released into the synaptic cleft when an action potential at the axon terminal induces communication between neurons at highly specialized junctions known as synapses. These chemical signals then bind to specific receptors on the postsynaptic membrane

depending on the type of neurotransmitter, in order to excite or inhibit the receiving cell, thus changing neuronal activity patterns (Hara, 2024).

Among the most well-characterized neurotransmitters are dopamine, which plays a central role in reward processing and motivation; serotonin, which is implicated in mood regulation; and glutamate, acknowledged as the central nervous system's main excitatory neurotransmitter (Zhou, *et al.* (2023).

2.3 Brain electricity

Neurons transmit electrical signals that facilitate communication and enable complex functions such as perception, movement, and recognition, which are fundamental to brain activity (Nelson, (2017)). They communicate via action potentials, which are swift alterations in the electrical potential across the neuron's membrane. These potentials action occur when the membrane depolarizes beyond a specific threshold, resulting in the activation of voltage-gated ion channels. As a consequence, sodium (Na+) ions enter the cell, causing depolarization, while potassium (K+) ions exit, leading to repolarization. This creates a wave-like electrical signal that travels along the axon toward the synaptic terminal. Upon reaching the synapse, neurotransmitters are released into the synaptic cleft and attach to receptors on the subsequent neuron, thereby continuing the transmission of the signal (Raghavan, *et al.* (2019). Furthermore, electroencephalography (EEG) captures the brain's electrical activity and the synchronized firing of extensive groups of neurons, offering valuable insights into neurological disorders, sleep patterns, and states of consciousness (Lin and Wu, (2024)).

2.4 Epilepsy

A class of long-term neurological conditions known as epilepsy is defined by frequent episodes of aberrant brain neuronal activity. Clusters of neurons fire excessively and concurrently during a seizure, often at 500 signals per second, which is far greater than the typical firing rate of neurons (Fenoglio, *et al.* (2013). A number of clinical manifestations may result from this abrupt spike in synchronized electrical activity, which might interfere with normal brain function, including involuntary motor activity, altered sensory perception, emotional disturbances, and abnormal behaviors. In many cases, it may also result in a transient loss of consciousness (Shorvon, 2011).

2.5 Causes of Epilepsy

Epilepsy can have many different causes, including acquired and genetic factors. Genetic mutations are a significant contributor, for example, in severe childhood epilepsy, mutations in the CACNA1E gene cause calcium channels in neurons to be disrupted, which result in excessive electrical activity and seizures (Helbig and Tayoun, (2021). Some epileptic adult, including temporal lobe epilepsy, are also influenced by somatic mutations that develop after conception. Certain genetic pathways, such as the RAS/MAPK pathway, which is also linked to cancer, may be impacted by these mutations, opening up new therapeutic options. (Bridger, (2023)). Additionally, research has identified the TMEM184B gene as a possible factor in epilepsy, as its absence or alteration can cause neurons to fire excessively, affecting normal neural communication. This finding emphasizes how ion channel and neuron excitability dysfunctions may be the cause of some types of epilepsy (Kelley, (2022)).

2.5.1 Genetic cause (primary epilepsy)

Mutations in particular genes that impact synaptic functioning, neuronal signaling, and brain development are linked to genetic causes of epilepsy, developmental and epileptic encephalopathies (DEE), a severe group of epilepsy disorders, are linked with over 100 genetic mutations, including those in the SCN1A and STXBP1 genes. These mutations cause seizures and associated neurological abnormalities by interfering with cellular functions like calcium signaling and synaptic activation. (Mefford and Guerrini, (2012)). Moreover, malformations of cortical development (MCD) and focal epilepsies have been linked to somatic brain mutations, which happen after birth. These mutations highlight the intricate interaction between genetic and developmental factors by affecting genes involved in calcium signaling and cortical development. (University of California, San Diego. (2022).

2.5.2 Acquired cause of epilepsy (secondary epilepsy)

Numerous conditions that impact the structure and function of the brain can result in epilepsy. Because they can cause aberrant electrical activity in the brain, neurological disorders like stroke, traumatic brain traumas, and brain tumors are important causes of epilepsy. Seizures can also be caused by illnesses that inflame the brain or surrounding tissues, such as encephalitis or meningitis (Hashemian, *et al*., (2021). Another component may be autoimmune disorders, in which the brain is mistakenly attacked by the immune system, resulting in epileptic episodes. Additionally, epilepsy has been connected to vascular abnormalities and degenerative diseases like Alzheimer's disease especially in older people. These secondary causes frequently draw attention to epilepsy as a sign of more serious underlying problems (Balestrini, (2021).

Impaired kidney function can also lead to seizures through several mechanisms, primarily due to the accumulation of toxins and imbalance metabolism. The retention of uremic solutes causes cerebral impairment in uremic encephalopathy, a disease that arises from kidney failure. This accumulation may result in seizures via changing the action of neurotransmitters, such as by raising excitatory amino acids like glycine and lowering inhibitory neurotransmitters like gamma-aminobutyric acid (GABA). (Olano, *et al.,* (2024)). Additionally, electrolyte imbalances, including disturbances in calcium, magnesium, and sodium levels, are common in kidney dysfunction and can lower the seizure threshold. Uremic encephalopathy and related seizures are more likely to occur when kidney disease worsens, especially when the estimated glomerular filtration rate (eGFR) drops below 15 mL/min. (Dhondup and Qian, (2017). Furthermore, dialysis disequilibrium syndrome, which is occasionally brought on by abrupt changes in uremic toxins during treatment, can result in neurological symptoms, including seizures. (Lim, *et al.,* (2020).

Through many processes, impaired glucose levels—whether from hypoglycemia or hyperglycemia—can significantly increase the risk of seizures. In hypoglycemia, the brain's primary energy source is depleted, which results in neuronal dysfunction. (Fusco, *et al.,* (2023). As blood glucose falls below 2.5 mM, the cerebral metabolic rate for glucose drops more rapidly than oxygen consumption, resulting in energy deficits. This lack of energy disrupts the modulation of neurotransmitters, specifically raising glutamate levels and lowering GABAergic inhibition, which can lower the threshold for seizures. (Siegel, *et al.,* (2011)). On the other hand, hyperglycemia can also make people more susceptible to seizures, particularly when it occurs in situations like non-ketotic hyperglycemia. Elevated blood glucose levels can lead to osmotic changes, causing neuronal dehydration and electrolyte imbalances. These disruptions increase neuronal

excitability by affecting ion transport and membrane potential. Hyperglycemia can also decrease the availability of the inhibitory neurotransmitter GABA, which lowers the seizure threshold even more. (Chan and Lim, (2017).

Nocturnal seizures, or seizures that happen during sleep, are a significant concern for individuals with epilepsy. These seizures often arise during non-Rapid Eye Movement (NREM) sleep stages, particularly stages 1 and 2, and are less common during REM sleep. (Malow, *et al.,* (2002)). The transition between sleep stages can influence seizure activity; for instance, the shift from wakefulness to sleep and between different NREM stages may alter cortical excitability, potentially triggering seizures. (Pavlova and Dworetzky, (2021). Notably, sleep affects the propagation of seizures because, when asleep, temporal lobe seizures are more likely to develop into generalized tonic-clonic seizures than when awake. (Herman, *et al.,* (2001). Furthermore, disorders like sleep apnea can make seizures more frequent and severe, highlighting how crucial it is to treat sleep-related problems when managing epilepsy. (Malow, (2004).

2.6 A seizure

An abrupt spike in aberrant brain activity that interferes with regular neuronal communication is called a seizure. These can range from severe convulsions to momentary unconsciousness. Epilepsy, head trauma, infections, high temperature (febrile seizures), and genetic predispositions are among the common causes. (Engel, (2013)).

Seizures can be classified as either focal, which start in one area of the brain, or generalized, which affect both hemispheres at once. Flashing lights, stress, alcohol, and lack of sleep are some examples of triggers. Depending on the kind and degree, treatments

can include medication, lifestyle modifications, or, in certain situations, surgery. (Shorvon, (2005)).

2.7 The different kinds of seizure

2.7.1 Focal seizure

Focal seizures, also known as partial seizures, originate in one portion of the brain and can spread to other parts of the brain. They fall into two primary categories: focal impaired awareness seizures, in which consciousness is lost, and focal aware seizures, in which consciousness endures are the two primary categories into which they fall. Unusual feelings, movements in a particular body area, or repetitive motions or lip-smacking are examples of symptoms.

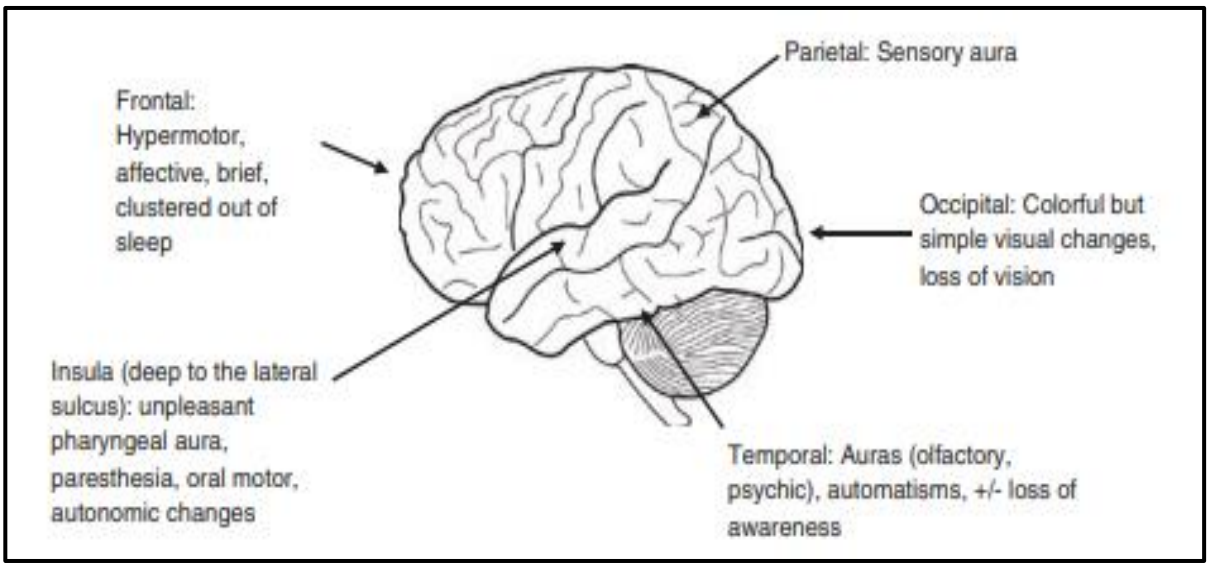


Fig. 2-1 Seizure semiology by lobe *(Kanner and Ribot, (2025)*

These seizures can occasionally spread to involve the whole brain, leading to generalized tonic-clonic seizure. After seizure spread occurs, signs from the symptomatic

zone—clinical symptoms caused by electrical stimulation of a brain region—may appear rather than the epileptogenic zone, which is where the seizure originated. (figure 2.1) (Chaudhary and Herlopian, (2016)). Brain injuries, strokes, tumors, and developmental abnormalities are among the common causes of focal seizures. Infections like meningitis, diseases like dementia, and genetic predispositions are also considered risk factors. (Khan and Cascino, (2023).

- Frontal

focal seizures that start in the frontal lobe are characterized by sudden and frequently dramatic symptoms because the frontal lobe is involved in behavior and motor control. Vocalizations, strange postures, and hyperkinetic movements like thrashing or pelvic thrusting can all be signs of these seizures. They can happen in groups, especially at night, and are frequently short—less than two minutes. The symptoms can be confused with other conditions such as sleep problems or non-epileptic episodes and vary depending on which part of the frontal lobe is affected such as the main motor cortex or supplementary motor region. (Koutroumanidis, *et al,.* (2014)).

- Temporal

Depending on the specific area affected, focal seizures that originate in the temporal lobe may exhibit a variety of symptoms. Temporal lobe epilepsy (TLE), recognized as the most common form of focal epilepsy, can involve both the medial and lateral regions of the temporal lobe. Individuals experiencing these seizures might encounter intense feelings of fear or excitement, confusion, or déjà vu, as these episodes frequently alter perception, memory, and emotions. (Van Paesschen, *et al.,* (2001). Medial temporal lobe epileptic seizures (MTLE) typically begin in the hippocampus, leading to

symptoms such as repetitive movements, lip-smacking, or unusual sensations. In these instances, patients may remain conscious but possess a reduced level of awareness.

(Engel, *et al.,* (2003).

- Parietal

Focal seizures that begin in the parietal lobe are relatively rare but can manifest in a variety of ways depending on the specific brain regions affected. Seizures that start in the parietal lobe can result in proprioception and spatial orientation problems, as well as feelings of numbness, tingling, or heat. This is because the parietal lobe is essential for sensory processing, spatial awareness, and the integration of sensory information. Patients may occasionally suffer from sensory hallucinations, such as experiencing an electric shock-like sensation or having different touch impressions. (Sun, *et al,.* (2019).

- Occipital

Focal seizures originating in the occipital lobe are relatively rare and involve disruptions in the brain's visual processing centers. These seizures can be manifested as visual hallucinations, such as flashes of light, blurred vision, or even complete visual loss (amaurosis), often affecting one side of the visual field depending on the location of the seizure within the occipital cortex (Defeating Epilepsy Foundation. (2021). Due to overlapping symptoms, such as visual problems, seizures are sometimes mistaken with migraines and last vary from a few seconds to several minutes. (Duncan, (2015).

- Insular

Insular seizures are a focal seizure that originate in the insular cortex, because of the insular cortex many connections to other areas of the brain can manifest with a wide range of symptoms. Autonomic symptoms like heart rate changes, excessive sweating, or feelings of warmth can be brought on by these seizures. Patients may also feel constricted in their throats and have laryngeal discomfort, as well as sensory problems like tingling or soreness. It can be difficult to diagnose insular seizures since their symptoms frequently match with those of seizures from adjacent regions, such as the temporal or frontal lobes. The insular cause of seizures is usually identified using advanced neuroimaging and EEG studies. (Jobst and Gonzalez-Martinez. (2019)).

2.7.2 Generalized seizure

Both hemispheres of the brain are involved in generalized seizures, which frequently result in unconsciousness. They are divided into a number of categories, including atonic seizures, which result in a sudden loss of muscular tone and lead to falls; tonic-clonic seizures, cause muscles to tighten and jerk; and absence seizures, manifest as transient lapses in awareness. Brain damage, underlying illnesses including infections, or genetic reasons may cause these seizures. Common symptoms include convulsions, muscle rigidity, and temporary confusion. (Moshé, *et al.,* (2015).

They can be further divided into several subtypes, each with distinct characteristics:

Table (2.1): type of generalized seizure:

Type of Generalized Seizure	Description
Tonic-Clonic	Characterized by rhythmic jerking (clonic) and stiffening (tonic) muscular movements.
Absence	Abrupt losses in consciousness, frequently accompanied by blinking or subtle muscular motions like gazing.
Myoclonic	Muscle or set of muscles jerking briefly, usually bilaterally, like a shock .
Atonic	Sudden loss of muscle tone, leading to collapse or head drooping.
Tonic	Sudden muscle stiffening, which can impair breathing or cause falls.
Clonic	Repeated rhythmic jerking movements, often affecting the arms, neck, or face.

This classification is based on a detailed system that identifies seizure types by motor and nonmotor symptoms as well as by EEG findings. (Fisher, *et al.* (2017).

2.8 The difference between epilepsy and seizure

While seizures and epilepsy are interconnected, they are fundamentally different clinical concepts. A seizure refers to a short episode of abnormal electrical activity in the brain, which can be manifested in various symptoms, including loss of consciousness, convulsions, or altered sensory perceptions. Seizures can occur as isolated incidents triggered by factors such as head injuries, high fevers, electrolyte imbalances, or adverse reactions to medications. Notably, experiencing a single seizure does not automatically imply the presence of epilepsy (International League Against Epilepsy (ILAE) and International Bureau for Epilepsy (IBE) (2005)).

In contrast, epilepsy is a chronic neurological disorder characterized by a recurring and unpredictable tendency to experience seizures. A diagnosis of epilepsy typically follows established criteria, requiring an individual to have two or more unprovoked seizures occurring at least twenty-four hours apart. The causes of epilepsy may involve structural brain injury, genetic factors, or neurodevelopmental issues (Fisher, *et al.,* (2014)).

2.9 Basic Characteristics of epilepsy

2.9.1 provoked seizure

A provoked seizure, also called an acute symptomatic seizure, is a seizure that happens directly in response to a temporary brain condition, such as metabolic imbalances, infections, or head injuries; common causes include exposure to toxins, traumatic brain injuries, infections such as meningitis or encephalitis, or a significant drop in blood sugar (hypoglycemia) or a high fever in children. (Chen, *et al.* (2014). Furthermore, provoked seizures are directly linked to an identifiable and frequently reversible cause,

and treatment focuses on treating the underlying condition to prevent further seizures. (Shorvon and Guerrini (2016)).

2.9.2 Unprovoked seizure

An uncontrolled electrical disruption in the brain without a known, immediate cause is known as an unprovoked seizure. Such seizures can occur spontaneously and are not triggered by certain stimuli (Berg and Shinnar (2020). A person may be diagnosed with epilepsy if he has two or more unprovoked seizures, however having just one unprovoked seizure does not necessarily indicate the someone has epilepsy. (Fisher, *et.al.* (2014)). Following a first unprovoked seizure, the chance of recurrence varies; research suggests that the probability of having another seizure within five years is between one-third and one-half. A number of variables, such as underlying brain abnormalities, EEG results, and family history, affect its management and prognosis. (Hauser and Beghi, (2008)).

2.10 Tetranectin

Tetranectin is a plasma protein linked to the fibrinolytic system that has shown promise as a biomarker for a number of illnesses, it has gained attention as a biomarker due to its diverse roles in physiological and pathological conditions. (McDonald, *et al.,*(2020)).

2.10.1 Tetranactin clinical relevance:

- **Cancer Diagnostics and Prognostics**: In oncology, tetranectin has demonstrated promise as a biomarker. Reduced plasma tetranectin levels have been linked to the advancement of cancer, especially when metastases are present. Early diagnosis and disease progression monitoring may be facilitated by this protein's ability to distinguish between metastatic and non-metastatic tumors. (Chen, *et al.,* (2019).

- **In heart failure (HF):** Tetranectin levels, which are closely correlated with the severity of heart failure. Tetranectin's expression in cardiac tissue is associated with fibrotic processes, indicating that it contributes to heart remodeling and fibrosis. Because of this, it can be used as a biomarker for both diagnosis and as a possible target for treatment. (McDonald, *et al.,* (2020)).

- **In liver function:** Tetranectin levels have been found to fluctuate in cirrhosis and other liver-related disorders, which suggest that it may possibly operate as a marker for liver function. Its potential use in hepatology, particularly in evaluating fibrosis and regeneration. (Westergaard, *et al.,* (2003).

- **Reproductive Health:** Its function also extends beyond pregnancy, since study have examined its levels in preeclampsia and other disorders. It may have predictive value for problems and shed light on mother health and placental function throughout pregnancy. (Høgdall, *et al.,* (1991).

2.10.2 The Role of Tetranectin biomarker in epilepsy

Tetranectin, a protein involved in tissue remodeling and fibrinolysis, has been investigated for its potential role as a biomarker in neurological disorders, particularly epilepsy. (Sutton and Moore (2020).

Tetranectin levels in serum and cerebrospinal fluid (CSF) have been found to vary between epileptics and healthy controls. Epileptic individuals had lower serum tetranectin levels and higher CSF tetranectin levels. Drug-refractory epilepsy exhibits these differences more markedly, indicating that tetranectin may be a useful biomarker for diagnosing epilepsy and its treatment-resistant variants. (Wang, et al. (2010).

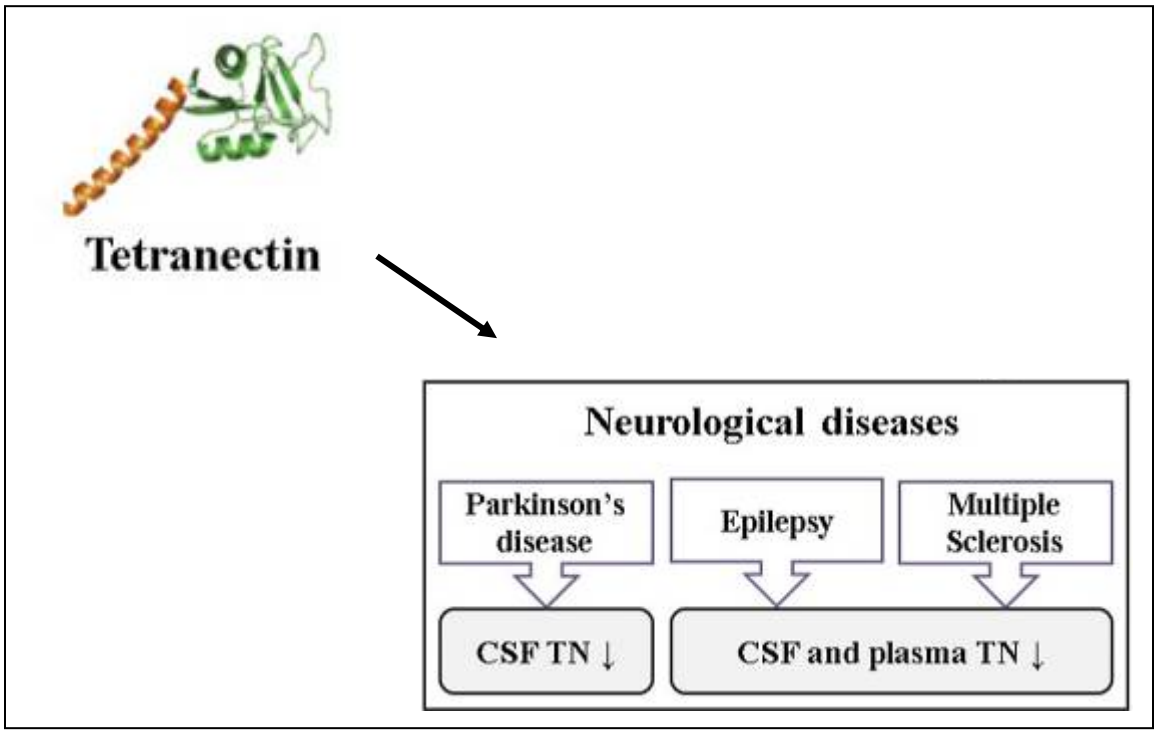


Fig.2.2 tetranectin in neurological diseases (Zhang, *et al.,* (2023).

2.11 protein S100B

The calcium-binding protein S100B is predominantly secreted by astrocytes in the CNS. It has both intracellular and extracellular regulatory functions, thus it serves as an important biomarker in several types of neurological diseases. In clinical protocols, S100B blood levels have been considered the gold standard to assess the severity of TBI, representing an ideal marker of astrocytic activation or injury. In TBI management, its use as scouting and prognostic tools has been beneficial for the prediction of patient outcome, and it can help guiding therapeutic decisions (Mondello, *et al.,* 2017).

Protein S100B plays a role in the pathophysiology of epilepsy, in addition to traumatic brain injury (TBI). Under normal physiological conditions, S100B is known to promote neuronal survival, stimulate neurite outgrowth, and provide neuroprotection. However, during pathological events such as seizures, its extracellular concentration rises significantly, leading to functional changes. At elevated (micromolar) levels, S100B triggers a pro-inflammatory signaling cascade by interacting with receptors like TLR-4 (Toll-like Receptor 4) and RAGE (Receptor for Advanced Glycation End Products) (Bianchi, *et al.,* (2010). These pathways ultimately result in neuronal injury and loss, involving the activation of NF-κB, increased generation of reactive oxygen species (ROS), and the release of pro-inflammatory cytokines, including IL-1β and inducible nitric oxide synthase (iNOS) (Seçen, *et al.,* (2023).

Several investigations have found that individuals with epileptic seizures had higher levels of S100B, indicating that S100B is more than just a passive indicator of neuronal injury., but an active participant in the neuroinflammatory environment associated with epilepsy (Vezzani and Friedman, (2011). In addition, recent investigations have detected S100B in alternative biofluids, such as saliva, indicating its potential as a non-invasive biomarker for CNS disorders. This approach provides a more accessible and

less invasive sampling method compared to traditional blood draws (Kayaba, *et al.,* (2022).

Given its involvement in inflammatory cascades, S100B also represents a promising therapeutic target. Strategies aimed at modulating S100B expression or blocking its interaction with RAGE may help attenuate neuroinflammatory responses and reduce seizure-induced neuronal injury. Such interventions could open new avenues for therapeutic development, with the goal of minimizing seizure frequency and severity, ultimately improving the quality of life for individuals living with epilepsy.

(Michetti, *et al.,* (2012).

2.12 High-mobility group box 1 (HMGB1)

High-Mobility Group Box 1 (HMGB1) is a nuclear protein with well-established roles in chromatin architecture and gene regulation, but it also functions extracellularly as a key mediator of inflammatory responses. Owing to its involvement in various inflammatory and neurodegenerative processes, HMGB1 has emerged as a promising biomarker and potential therapeutic target in a range of neurological disorders, including traumatic brain injury (TBI), epilepsy, cognitive dysfunction, and neuroinflammation (Yang *et al.,* 2018).

The pathophysiology of numerous important illnesses, including Parkinson's disease, stroke, autism spectrum disorder, depression, multiple sclerosis, and amyotrophic lateral sclerosis, has been linked to HMGB1 in the setting of central nervous system pathology, further reinforcing its utility as a common pathological mediator across neuroinflammatory diseases (Paudel *et al.,* 2022).

In Alzheimer's disease, HMGB1 exacerbates cognitive decline by binding to amyloid-beta (Aβ) plaques, thereby hindering their clearance by microglial phagocytosis and promoting sustained neuroinflammation (Takata *et al.,* 2004). Similarly, in Parkinson disease, HMGB1 is released from the nucleus into the cytoplasm of dopaminergic neurons, particularly within the substantia nigra, where it interacts with alpha-synuclein aggregates in Lewy bodies. This interaction impairs autophagic mechanisms, contributing to the progression of neurodegeneration (Shao, *et al.* (2019).

Taken together, these findings highlight HMGB1 as a critical molecular link between neuroinflammatory signaling and neuronal injury, suggesting its potential value not only in diagnostic and prognostic applications but also as a target for therapeutic intervention across a broad spectrum of neurological disorders.

In multiple sclerosis, increased HMGB1 levels in serum and cerebrospinal fluid are seen in patients, and these levels are correlated with relapse rates and disease activity. By interacting with Beclin-1 to facilitate autophagy, HMGB1 may increase neuroinflammation. (Malhotra, *et al.* (2015).).

In amyotrophic lateral sclerosis (ALS), Glial cells have an increased HMGB1 that interacts with receptors like TLR2, TLR4, and RAGE to release pro-inflammatory cytokines and cause motor neuron degeneration. (Paudel, *et al.,* (2020).

In diabetes-associated cognitive decline, HMGB1 activates the NLRP3 inflammasome, initiating pyroptosis in vascular cells and contributing to blood-brain barrier breakdown and cognitive impairment. (Liu, *et al.,* (2021).

In diabetic nephropathy, changes in renal function are correlated with higher serum levels of HMGB1. It is a promising biomarker for early detection that can be used to monitor the progression of disease. (Peng, *et al.* (2024).

2.12.1 The Role of HMGB1 biomarker in epilepsy

A noteworthy biomarker for epilepsy has been identified as High-Mobility Group Box 1 (HMGB1), especially when it comes to drug-resistant cases. Drug-resistant epilepsy patients have significantly higher blood levels of HMGB1 than both healthy people and patients with well-controlled, drug-responsive seizures (Walker, *et al.,* (2022).

Moreover, the identification of specific HMGB1 isoforms, such as acetylated and disulfide HMGB1, has been closely associated with drug resistance and epileptogenesis. These isoforms are believed to play mechanistic roles in sustaining chronic neuroinflammation and seizure recurrence, thereby offering additional value as pathophysiological markers of treatment-refractory epilepsy (Maroso *et al.,* 2016).

2.13 HBA1C

One indicator of long-term blood glucose levels, hemoglobin A1c (HbA1c), has been linked to epilepsy treatment and prognosis. Increased seizure intensity and recurrence have been linked to elevated HbA1c levels, which are a sign of inadequate glycemic control. In research, individuals with hyperglycemia diabetes who had their first seizure had significantly higher HbA1c levels if they had another seizure (11.8% vs. 8.6%, $p<0.05$) than those who did not. Additionally, patients who had HbA1c levels higher than 9% were more likely to experience seizure clustering and recurrence. (Singhal, *et al.,* (2008).

Seizures can also occur in individuals with low hemoglobin A1c (HbA1c) levels, primarily due to hypoglycemia. Hypoglycemia, defined as blood glucose levels falling below normal, can lead to neurological symptoms, including seizures. While seizures are relatively uncommon, they are more likely to happen when glucose levels fall

significantly. A study found that generalized tonic-clonic seizures occurred when serum glucose levels fell below 2.0 mM, and focal seizures were noted at glucose levels as high as 3.3 mM. (Halawa, *et al.* (2014). Additionally, lower HbA1c levels have been linked to a higher risk of severe hypoglycemia and hypoglycemic coma, which includes seizures, in young patients with type 1 diabetes. But with time, patients with lower HbA1c levels had a lower relative risk of severe hypoglycemia, maybe due to improved management strategies. (Karges, *et al.* (2014). Furthermore, HbA1c has been investigated as a possible biomarker for tracking systemic ketosis and diet adherence in individuals with drug-resistant epilepsy on ketogenic diets. Higher blood ketone levels were linked to lower HbA1c levels, indicating its potential use in the management of such dietary treatments. (de Haas, *et al.,* (2022).

2.14 Urea and creatinine

Serum urea, commonly known as blood urea nitrogen (BUN), is a metabolic waste product generated in the liver through the breakdown of proteins and subsequently eliminated by the kidneys. The assessment of serum urea concentrations is utilized to evaluate kidney function and the process of protein catabolism. Normal serum urea levels typically range from 2.5 to 7.1 mmol/L (equivalent to 7 to 20 mg/dL), although these values may vary slightly among different laboratories. (Butt and Rodan, (1990).

Seizures and epilepsy have been linked to elevated levels of urea and creatinine, two important markers of renal function. The study have shown that seizures occur in approximately 10% of patients with chronic renal failure (Capasso and Di Iorio (1994).

Seizures can result in acute kidney injury (AKI), especially if they are followed by rhabdomyolysis, a disorder in which muscle breakdown causes myoglobin to be

released into circulation, which may harm the kidneys. Patients may exhibit markedly elevated serum creatinine levels in certain situations, which would suggest compromised renal function. (Litchfield, *et al.,* (2014).

Prolonged seizures have also been linked to hyperuricemia, which is defined by increased serum uric acid levels. This illness can worsen renal impairment by causing acute uric acid nephropathy. (Abdulkader, *et al*. (2019).

2.15 serum calcium (Ca 2+)

Many physiological functions in the human body depend on calcium ions (Ca^{2+}). They function as a second messenger in a variety of cellular processes and are essential to signal transduction pathways. Ca^{2+}, for example, is essential for muscle contraction, fertilization, and the release of neurotransmitters from neurons. Numerous enzymes, particularly those involved in blood coagulation, need calcium ions as cofactors. (Brini, *et al.* (2013).

Calcium ions (Ca^{2+}). are essential to the pathophysiology of epilepsy because they are crucial for neuronal excitability and synaptic transmission. The influx of Ca^{2+}. into neurons is facilitated by voltage-gated calcium channels (VGCCs), which affect the release of neurotransmitters and the dynamics of action potentials. Characteristics of epileptic activity, neuronal hyperexcitability, can result from malfunction or alterations in these channels. For example, because of their function in thalamocortical oscillations, T-type calcium channels are linked to the development of absence seizures.

(Gourfinkel-An*, et al.* (2014).

The equilibrium between neuronal excitation and inhibition can also be upset by changes in calcium signaling pathways, which increases the risk of seizures. (Schwaller, (2014). On the other hand, hyperexcitability and the synchronization of neuronal firing might result from an increased influx of Ca2+ into neurons during a seizure. By upsetting the delicate balance between excitatory and inhibitory impulses in the brain, this aberrant calcium signaling helps to trigger epileptic activity. (Kawakami and Inoue, (2020).

Furthermore, chronically elevated calcium levels can cause excitotoxicity, neuronal death, and cellular damage, all of which may exacerbate epilepsy's long-term consequences. Calcium dysregulation is therefore a possible target for therapeutic therapies meant to regulate epileptic activity because it plays a significant role in both the acute and chronic phases of seizures. (Liu and Zukin, (2007).

Chapter Three

Materials

and

Methods

3.1 Materials

3.1.1 Apparatus analysis and equipment

The apparatus and equipment used in this work are listed in table (3-1).

Table (3-1): The apparatus and equipment

NO	Apparatus and Equipment	Company	Origin
1	Bench Centrifuge	Rotofix 32 A (Hettich)	Germany
2	Eppendorf tube	The feel-good company	USA
3	Gel tube	Xinle	China
4	Micropipette	Slamed	Germany
5	Refrigerator	Kiriazi	Egypt
6	Cotton	Al Salama	Iraq
7	EDTA tube	Vacuum Blood Collection Tube	China
8	Tourniquet	Voltaren	China
9	Syringe	Dolphin	Syria
10	Blue tips	Trust lab	China
11	Abbotte	Architect	Germany
12	ELISA reader	Accu Bio Tech	China
13	spectrophotometer	Agilent Technologies	USA

3.1.2 Kits

Kits were used according to the manufacturer and origin, as shown in the following table (3-2).

Table (3-2): the kits:

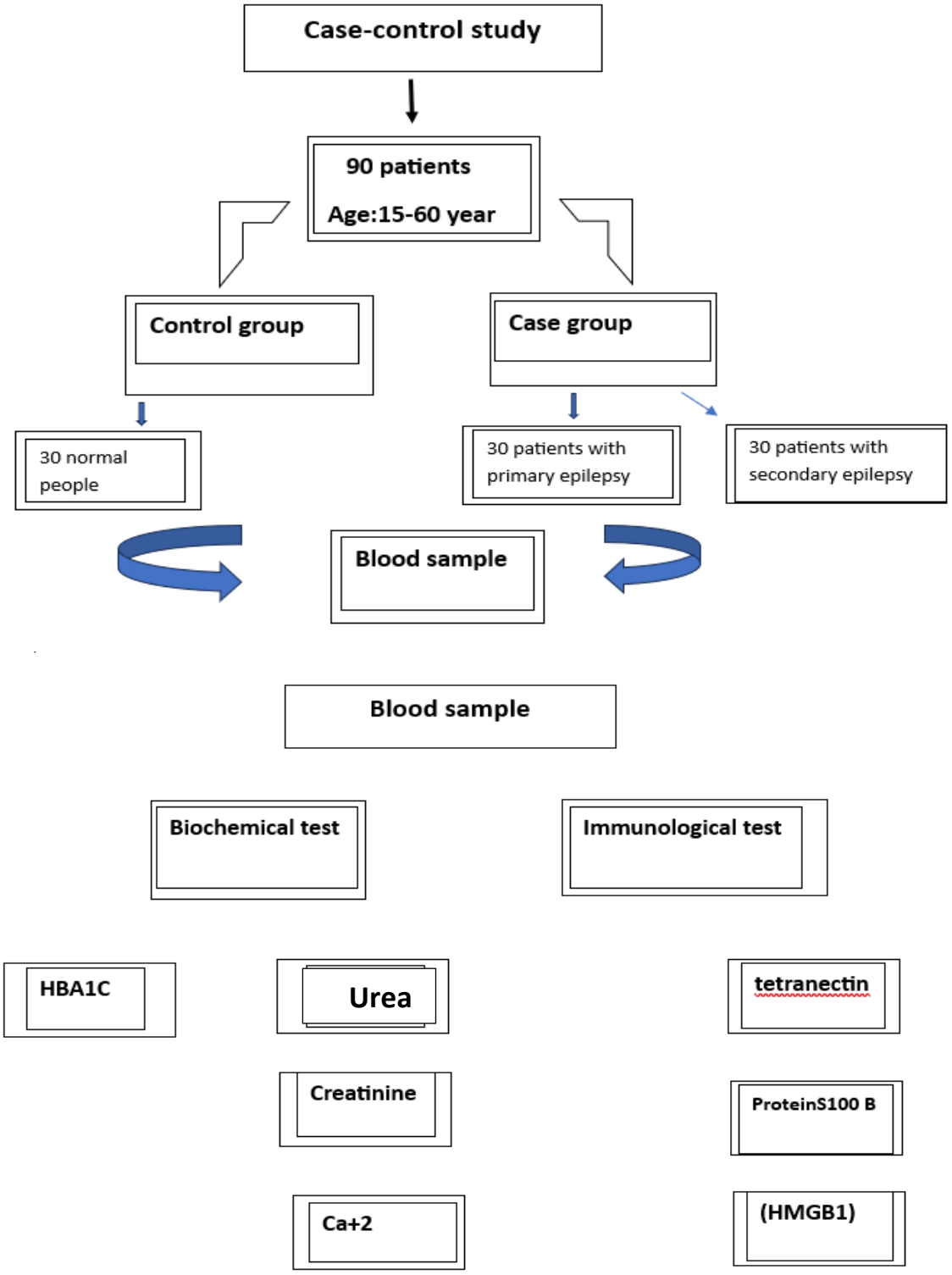
NO	Kits	Origin
1	tetranectin Kit	China
2	ProteinS100 B Kit	China
3	HMGB1 Kit	China
4	HBA1C Kit	U.K.
5	urea Kit	PG Italy
6	creatinine Kit	France
7	Calcium Kit	France

3.2 Methods

3.2.1 Study design

To determine the serum level of 3 promising biomarkers: Tetranectin, Protein S100B and High-Mobility Group Box 1 (HMGB1) on patients with primary and secondary epilepsy, a case-control design was employed in this study. It took place in the Karbala Province from November 2024 to April 2025. The clinical trial was approved ethically by the Ministry of Health, Iraq together with the Faculty of Applied Medical Sciences Ethics Committee, justifying authors' adherence to human ethical standards for clinical research (figure 3-1).

Figure (3-1): the study design:



3.2.2 Study population

A total of 90 individuals were enrolled in this study, comprising 30 patients with primary epilepsy, 30 patients with secondary epilepsy, and 30 healthy controls. Patient recruitment was conducted at Imam Hussein Teaching Hospital in Karbala, while control samples were obtained from community members and medical staff to ensure appropriate matching and variability. All participants provided informed consent prior to inclusion in the study.

The following were the study groups:

1. Patients: consisted of
   A) 30 patients with primary epilepsy
   B) 30 patients with secondary epilepsy

2. Control: consisted of 30 healthy people.

Consultant physicians selected epilepsy patients for diagnosis. After obtaining permission from each person participant in the research, an interview was conducted, and all notes and information were entered into a questionnaire (appendix). In addition to obtaining a patient's history, doing a clinical examination, and taking blood to test HBA1C and electrolytes linked to seizure.

3.2.2.a Inclusion criteria

1- Age :15-60 years.
2- Patients have seizure, primary epilepsy or secondary epilepsy.
3- A personal approval to participate in the study.
4- A person between the ages of 15 and 60 with no history of epilepsy or seizure.

3.2.2.b Exclusion criteria

Patients were excluded if they had:

1- Age under 15 years old or above 60 years old.
2- Patients with cancer anywhere in the body other than the brain.

3.2.3 Sample collection

From each participant, around 5 mL of venous blood was drawn. Gel and EDTA tubes were used to hold blood samples. The samples were centrifuged 5000 rpm for 10 minutes to extract the serum after allowing the blood in the gel tubes to coagulate. This serum was then utilized for the biochemical analysis of HbA1c, calcium Ca^2 and urea, and creatinine. The remaining portions of the serum were aliquoted into Eppendorf tubes and stored at -20 °C until further analysis. All samples were securely frozen in the hospital's laboratory freezer to preserve stability. Subsequent analysis of the targeted biomarkers; Tetranectin, S100P and HMGB1 was performed using a commercially available ELISA instrument in accordance with the manufacturer's protocols.

3.2.4 Ethical Management of Studies

University of Kerbala College of Applied Medical Science's Ethical The project was approved by the committee. Reference No: CLAMSKU/1. Every participant in this study was informed and gave their verbal agreement to participate prior to sample collection.

3.2.5 Measurement of Body Mass Index

The following equation was used to determine body mass index:

BMI = Weight (kg)/Height (m^2)

The present discourse examined the Body Mass Index (BMI) and its associated concerns, pathological conditions, and clinical relevance.

It was vital to comprehend the existing threshold values for each BMI category as established by the World Health Organization (WHO), which provides the following delineations:

- Underweight: 16.0–18.0 kg/m^2;
- Normal weight: 18.5–24.9 kg/m^2;
- Overweight: 25.0–29.9 kg/m^2
- Severe Underweight: < 16 kg/m^2
- 30.0 to 34.9 kg/m^2 is considered moderately obese
- 35.0 to 39.9 kg/m^2 is considered very obese.

3.3 Method

3.2.1 detection of serum urea concentration

Principle

Concentration of urea was measured by the Urea Assay Kit, according to the manufacturer's protocolUrea Assay Kit (Urea Kit) to quantitatively determine the urea concentration in urine, serum, plasma, cell lysates or tissue homogenates. Absorbance readings of the samples were referred to a standard curve prepared with known urea concentrations at the time (processing was performed in a 96-well microtiter plate). Each sample and standard were also treated with urease enzyme, responsible for converting urea to ammonia (NH_3) and carbon dioxide (CO_2) for 10min.

The liberated ammonia reacted with a chromogen reagent in an alkaline medium to produce a blue-green chromophore. OD was determined spectrophotometrically in a microplate reader at a wave-length ranging from 580 to 630 run after a 30 min incubation. The intensity of the color was directly related to the amount of urea in the sample.

Urea levels were calculated by comparing sample OD values to those on the standard curve, which demonstrated linearity up to 50 mg/dL.

Procedure

To guarantee accuracy and repeatability of results, all urea standards and test samples were tested in duplicate or triplicate. Every assay run, a newly prepared standard curve was generated to calibrate urea concentrations and maintain consistency across experiments.

To account for background ammonia present in the samples particularly in those with elevated ammonia levels, 2 paired wells were prepared per sample one well was treated with the entire mixture of urease and ammonia reagent (+U), while the other well was treated with ammonia reagent alone, without urease (−U). This approach enabled the subtraction of background ammonia from total signal, ensuring that the measured absorbance accurately reflected urea-derived ammonia and not pre-existing ammonia in the sample matrix.

1. Useing a plate reader liquid handling equipment or a multichannel pipette, add 10 µL of each sample or diluted urea standard into individual wells of a 96-well microtiter plate. If background ammonia is to be measured, prepare paired wells for each sample: one for the complete reaction (+U) and one for the background control (–U).
2. Add, 100 µL of the Urease/Ammonia Reagent (+U) to each sample and standard well. To avoid foaming, mix completely but softly.
3. Note: For samples requiring background correction, add 100 µL of

Ammonia Reagent without Urease (–U) to one well of each paired sample set.

4. To enable the enzymatic breakdown of urea, incubate the plate at 37°C for 10 minutes..
5. A multichannel pipette or automated dispenser should be used to add 100 µL of the developing reagent to each well. Mix well, again being careful not to let bubbles develop.
6. Incubate the plate for an additional 30 minutes at 37 °C to allow complete color development.
7. Measure the optical density (OD) of each well at 580–630 nm using a standard microplate spectrophotometer. Record absorbance values and determine urea concentrations by comparison with the standard curve.

Calculation of Results

1. Calculate the mean absorbances for each standard and for each sample and control.
2. Background correction. mean zero standard should be subtracted from each standard and sample value including itself.
3. Graph the standard curve.
4. Deduct the absorbance of the urease-treated sample well (A+U) from the sample well absorbance without urease (A-U) to obtain the sample absorbance difference of a pair of wells (with and with-out urease). The baseline ammonia concentration in the sample is (A-U) sample and the combined urea & ammonia background concentration in the sample is (A+U) sample. The urea concentration is the origin of the absorbance difference (ΔA): (A+U) - (A -U) = (ΔA)
5. To ascertain the amount of urea contained in each sample, compare its absorbance values to the standard curve. Only use numbers that fall inside the standard curve's range.

3.2.2 Detection of serum calcium concentration

Principle

Total Calcium Serum, Urine, Plasma. This test uses the CPC (O-Cresol Phthalein Complexone) method to measure the total amount of calcium in the blood, urine, or plasma. In the presence of an alkaline condition, CPC produces a dark red complex with calcium. The absorbance of this complex at 570 nm is directly proportional to the concentration of calcium present in the sample procedure

Procedure

The Kenza 240TX uses a sophisticated analytical methodology for measuring

	Analyzer Automated	Manuals procedure
Reagents	R1 120 µL R2 120 µL	WR ,1000 µL
Stander ,Control, Sample	6 µL	25 µL

total calcium using the CPC (O-Cresol Phthalein Complexone) method. Calcium ions form a brown red complex with CPC in an alkaline solution. Absorbance of the complex is quantified spectrophotometrically at a wavelength of 570 nm. The test is performed at 37C° to achieve an optimal reaction condition and accuracy. The temperature must be maintained constantly as the dye's absorbance is temperature sensitive.

After five minutes of incubation at room temperature, compare the absorbance at 570 nm to the reagent blank.

The color remains steady for an hour when exposed to light.

To calculate this, do the following:

Plasma or Serum:

$$Result = \frac{\text{Abs (Assay)}}{\text{Abs (Stander)}} \text{ x concentration of standard}$$

3.2.3 Detection of serum creatinine

Principle

The Jaffe reaction, in which creatinine combines with alkaline picrate to generate an orange-red complex, provides the basis for the colorimetric measurement of creatinine. The wavelength range used to measure this reaction is 490–510 nm, typically at 490 nm. Notably, the method does not require any sample pretreatment.

Procedure

Manual method

Stand reagent and specimens at room temperature.

Working reagents (R1+R2)	1000ul
Specimen	100ul

Stir well. To ensure a steady temperature, do kinetic testing at 37°C.

Read absorbance A1 after 30 seconds, and then read absorbance A2 at 490 nm (490-510) against distilled water precisely 120 seconds later..

Each test tube contains blank water, a calibrator, controls, and assays as specimens.

1- Performances are validated with serum on KENZA 240TX using working reagent as manual method.
2- Use on other analyzers should be validated by users.
3- Applications validated on KENZA automated analyzers and proposal on other analyzers are available on request.
4- Dilute specimen (1+4) with saline over the linearity limit, then re-assay with dilution factor 5 in mind..
5- Specimen: demineralized water (blank), serum (1+19) in demineralized water before measurement.

CALCULATION

Serum or plasma

$$Result = \frac{(\mathrm{A2}-\mathrm{A1})Assay-(A2-A1)Blank}{(\mathrm{A2}-\mathrm{A1})Standerd-(a2-A1)blank} \text{ x Standard Concentration}$$

3.2.4 Detection of HBA1c

Principle

HbA1c and Hb in the sample mix with mouse anti-human HbA1c and Hb monoclonal antibodies when the sample is placed in the sample port on the test card. These antibodies are then attached to fluorescent particles to create fluorescent particles, antibody, and antigen complexes. The amount of HbA1c in the sample is directly connected to its fluorescence intensity. This immune complex travels down the nitrocellulose membrane to the test area (T) and interacts with the pre-coated mouse anti-human HbA1c monoclonal antibody. A quality control line is created when the remaining fluorescent antibody particle reaches the quality control area (C) and combines with the pre-coated goat anti-human Hb monoclonal antibody. The ratio of HbA1c to Hb was calculated by the fluorescence signal intensity. The test area (T) will not appear fluorescence, if the sample does not contain HbA1c.

Procedure

Before using, bring all reagents to room temperature (18–25°C).

(1) Startup: Click “STD Mode” in the main menu to enter the measurement interface, click “Item” to select the desired test item and click “Type” to select the sample type.

(2) Enter the card swiping interface by clicking "Lot No." Place the corresponding item's mag card in the magnetic induction zone; a "di" sound indicates

that the mag card has been successfully swiped. Make sure the mag card and the test card are from the same batch.

(3) Sampling: Add 10μL of whole blood into a centrifuge tube with 1000μL of the sample diluent, mix for 1 minute. Take 100μL diluted sample, drop vertically to the sample port directly on the test card and start timing.

(4) Insert it into the analyzer's test slot (the sample port ends toward the inside). Click "Measure", the instrument will detect and print out the results automatically after 15 minutes (If using "Fast Mode", keep it for 15 minutes and quickly insert into the analyzer's test slot)

3.2.5 Determination of Human Tetranectin

Principle

The ELISA test serves to detect a specific group of drugs in sample. Initially, a human CLEC3B-specific antibody is coated onto the wells of a microplate. When the sample is introduced, the immobilized antibody interacts with the CLEC3B antigen present in the sample. Subsequently, a biotinylated human CLEC3B antibody is added, which selectively binds to the CLEC3B antigen in the sample. After this, HPR-conjugated streptavidin is introduced, which exclusively reacts with the biotinylated antibody. A washing step is conducted to eliminate any unbound streptavidin-HRP after incubation. The addition of the substrate solution initiates a colorimetric reaction that is proportional to the concentration of human CLEC3B. Finally, an acidic stop solution is added to halt the reaction, and the absorbance is measured at 450 nm.

Reagent Preparation

•All reagents were at room temperature before use.

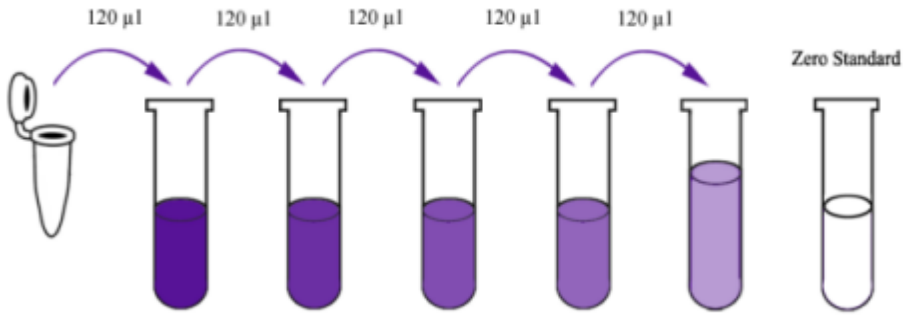
Reconstituted 120 µL of the 640 ng/ml standard by adding 120 µL of standard diluent, resulting in a 320 ng/ml standard stock solution. After the reconstitution, let the standard sit for 15 minutes, gently shaking it before any further dilution .

To create standard curves, a 1:2 serial dilution of the 320 ng/ml stock performed in the standard diluent. This will yield duplicate standards with concentrations of 160, 80, 40, and 20 ng/ml.

Any remaining standard solution was stored at -20°C and is stable for up to a month.

The dilution scheme for the standard solutions is as follows:

320(ng/ml)	Standard No.5.	120ul Original standard + 120ul standard diluent.
160(ng/ml)	Standard No.4.	120ul standard No.5 + 120ul standard diluent.
80(ng/ml)	Standard No.3.	120ul Standard No.4 + 120ul Standard diluent.
40(ng/ml)	Standard No.2.	120ul Standard No.3 + 120ul Standard diluent.
20(ng/ml)	Standard No.1.	120ul Standard No.2 + 120ul Standard diluent.



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/ml	320ng/ml	160ng/ml	80ng/ml	40ng/ml	20ng/ml

• 20 milliliters of wash buffer should be diluted. To make 500 milliliters of 1x Wash Buffer, concentrate 25 times into distilled water. If crystals have developed, stir gently until they have totally dissolved.

Procedure

1. All reagents were allowed to acclimate to room temperature prior to commencing the protocol.
2. The number of strips required for the assay determined and positioned in the appropriate frames, any unused strips stored at a temperature between 2 to 8°C.
3. 50 µL of the standard solution dispensed into the designated standard wells.
4. Introduce 40 µL of the sample into the sample well, followed by 50 µL of streptavidin-HRP into both the standard and sample wells, excluding the blank control wells. Then, add 10 µL of Human CLEC3B antibody and mix gently. Cover the plate with a sealer and incubate at 37°C for 1 hour.
5. After incubation, remove the sealer and wash the plate five times with wash buffer. Subsequently, wash each well three times with 300 µL of wash buffer per wash, allowing the wash buffer to sit in the wells for 30 to 60 seconds. Blot the plate dry using paper towels.
6. Add 50 µL of substrate solution A followed by 50 µL of substrate solution B to each well. Cover the plate with a new sealer and incubate in darkness at 37°C for 10 minutes.
7. After the incubation period, add 50 µL of stop solution to each well, which will cause an immediate color change from blue to yellow.
8. Measure the absorbance (OD) of each well at 450 nm using a microplate reader 10 minutes after the addition of the stop solution.

To calculate the findings, create a standard curve by graphing each standard's average optical density (OD) on the Y-axis against its corresponding concentration on the X-axis. Create the best-fit curve using these shown points.

Finding the best fit may be aided by regression analysis, and the most accurate and effective way to do this is by using computer-based curve-fitting software.

Typical Data

Only the E6262Hu standard curve is included here for demonstrative reasons. To guarantee precise quantification, a standard curve should be created for every batch of materials analyzed.

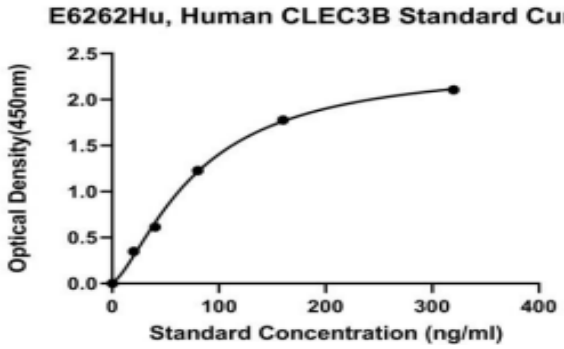


Figure (3-2) standard curve of human CLEC3B

3.2.6 Determination of Protein S100-B

Principle

Pretreatment of microplate wells with a human S100B antibody is included in an Enzyme-Linked Immunosorbent Assay (ELISA) kit. The immobilizedantibodies bind the S100B-containing species when latter is brought into contact with the surface. The biotinylation antibody Human S100B is an added and it binds to sample S100B antigen. After addition of the latter, streptavidin bound to HRP and conjugated to biotin is specifically bound to the biotinylated antibody. Streptavidin-HRP that did not bind to it is re-moved by washing after incubation. Addition of substrate solution results in a color reaction proportional to the concentration of Human S100B. The colorimetric determination is

stopped by addition of an acidic stop solution and the absorbance read at 450 nm.

Reagent Preparation

• All reagents should be at room temperature before use.

• Resuspend 120 microliter of the 320 nanogram/L standard using 120 micrliter of the standard diluent to create a 160 nanogram /L standard stock solution. Allow the reconstituted standard to incubate for 15 minutes while gently mix before proceeding with the dilutions. To establish the duplicate standard points, utilize the 160 nanogram /L stock to prepare a series of standard dilutions, calculating from 80 nanogram /L down to 10 nanogram /L in a 1:2 ratio: 40 nanogram /L, 20 nanogram /L, and 10 nanogram /L. Use any remaining mutagen within one month and store it at minus 20°C. The same procedure can be applied for the standard solutions.

160(ng/ml)	Standard No.5.	Original standard (120 ul) plus standard diluent (120 ul).
80(ng/ml)	Standard No.4.	120ul of standard diluent plus 120ul of standard no. 5.
40(ng/ml)	Standard No.3.	Standard No. 4 (120 ul) plus Standard diluent (120 ul).
20(ng/ml)	Standard No.2.	120ul of Standard Diluent plus 120ul of Standard No. 3.
10(ng/ml)	Standard No.1.	120ul of Standard Diluent plus 120ul of Standard No. 2.

• To make the Wash Buffer, dilute 20 milliliters of the 25× Wash Buffer Concentrate with 500 milliliters of distilled water, resulting in a 1× working solution. Mix gently and thoroughly until all crystals have fully dissolved.

Procedure

1. Handle the standards and samples according to the provided guidelines. Ensure that all reagents are brought to room temperature prior to use.
2. Determine the required number of strips for the assay and arrange them in the appropriate frames. Any excess strips should be stored at a temperature between 2 to 8°C.
3. Introduce 50 µL of the standard solution into the standard wells. **Note:** Do not incorporate any additional antibodies into these wells as the standard already contains a biotinylated antibody.
4. For the sample wells and standard wells (excluding the blank well), add 40 µL of the sample, 10 µL of the Human S100B antibody solution, and 50 µL of streptavidin-HRP. Gently mix the contents. After sealing the plate, incubate it for 60 minutes at 37°C.
5. After incubation, remove the seal and wash the plate five times using wash buffer. Each well should be washed three times with 300 µL of wash buffer, allowing it to soak for 30–60 seconds during each wash. After washing, either discard or suction off the wash buffer. **Note:** Wash five cycles using an automatic washer. Immediately dry the plate by patting with paper towels or another absorbent material.
6. Add 50 µL of substrate solution A and 50 µL of substrate solution B to each well. Once the fresh seal is applied, incubate the plate in the dark at 37°C for 10 minutes.
7. Add 50 µL of Stop Solution to each well, which will quickly change the color from blue to yellow.
8. Measure the optical density (OD) of each well at a wavelength of 450 nm using a microplate reader within 10 minutes after the addition of the stop reagent.

Calculation of Results

To create a standard curve, plot each standard's mean optical density (OD) values on the Y-axis against the corresponding concentrations on the X-axis. Create the best-fit curve using these data points. The best fit may be found via regression analysis, and the most effective and precise technique for this is computer-based curve-fitting software.

Typical Data: This is only an example of the typical curve for E3669Hu. Every set of samples analyzed requires a different standard curve to ensure precise quantification.

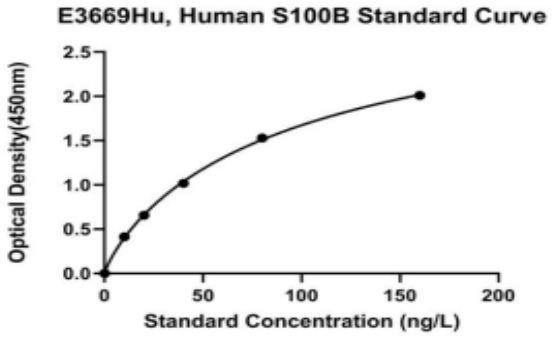


Figure (3-3) standard curve of human S100B

3.2.7 Determination of HMGB-1

Principle

A human HMGB1 antibody has been coated in the wells of the microtiter strips, respectively. This immobilized antibody binds the HMGB1 antigen in the sample on addition of same. The HMGB1 antigen then is captured by biotinylated Human HMGB1 Antibody. After this, biotin conjugated HRP is added, which is binding with the biotinylated antibody selectively.

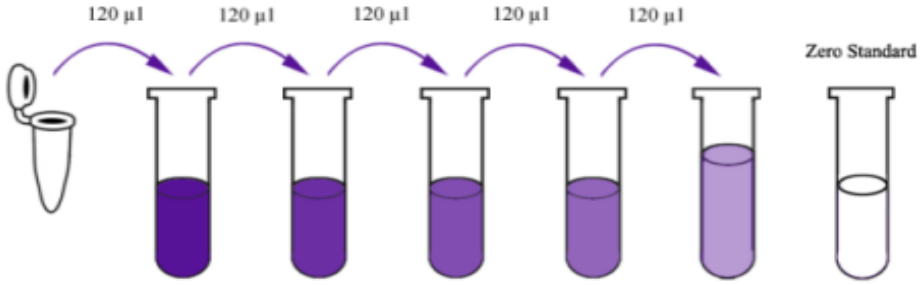
Unbound Streptavidin-HRP is removed after incubation by washing. The color change upon addition of the substrate solution depends on the quantity of Human HMGB1. An acidic stop solution is added to stop the reaction and absorbance is read at 450 nm.

Reagent Preparation

• Each and every reagent must be at room temperature.

• Reconstitute 120 µL of the 160 ng/ml standard with 120 µL of standard diluent to produce 80 ng/ml standard stock solution. Allow the solution to stand for fifteen minutes with occasional shaking before the dilutions are made. Dilute the 80 ng/ml stock point series 1:2 in standard diluent to successively prepare duplicates of the 40, 20, 10 and 5 ng/ml standard points. Unutilized solution should be used up within a month and stored at -20°C. For standard solutions, the dilution is in the same way:

80(ng/ml)	Standard No.5.	120ul Original standard + 120ul Standard diluent.
40(ng/ml)	Standard No.4.	120ul Standard No.5 + 120ul Standard diluent.
20(ng/ml)	Standard No.3.	120ul Standard No.4 + 120ul Standard diluent.
10(ng/ml)	Standard No.2.	120ul Standard No.3 + 120ul Standard diluent.
5(ng/ml)	Standard No.1.	120ul Standard No.2 + 120ul Standard diluent.



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
160ng/ml	80ng/ml	40ng/ml	20ng/ml	10ng/ml	5ng/ml

• Make a 1× working solution by diluting 20 mL of the 25× concentrated Wash Buffer with deionized water to a final volume of 500 mL. If there are crystals in the concentrate, mix them gently until they dissolve completely.

Procedure

1. Conduct the assay at room temperature.
2. Determine the number of strips required and place them in their respective frames. Store any unused strips at a temperature of 2-8°C.
3. Introduce 50 µL of the standard into the designated standard wells. **Note:** Avoid adding additional antibodies to these wells, as the standard already contains a biotinylated antibody.
4. Dispense 40 µL of the sample or standard (excluding blank control wells) into each well, followed by 50 µL of streptavidin-HRP and 10 µL of human HMGB1 antibody. Mix thoroughly. After sealing the plate, incubate for one hour at 37°C.
5. After removing the sealant, wash the plate five times using wash buffer. Perform automatic washing by soaking each well with 300 µL of wash buffer for 30-60 seconds per wash, then aspirate or decant each well.

After washing, dry the plate with tissue or other absorbent material.

6. Following the application of a fresh sealer, add 50 µL of substrate solution A and 50 µL of substrate solution B to each well, then incubate in the dark at 37°C for ten minutes.
7. Introduce 50 µL of stop solution into each well; the color will instantly shift from blue to yellow.
8. Measure the absorbance of each well at 450 nm using a microplate reader within ten minutes of adding the stop solution.

Calculation of Results

To create a standard curve, chart the average optical density (OD) of each standard on the vertical (Y) axis in relation to the concentration on the horizontal (X) axis. After plotting the points, draw a best fit curve. This optimal line can be determined through regression analysis, and the most effective tool for these calculations is computer-based curve-fitting software.

Typical Data: Only for demonstration purposes is the E1635Hu standard curve supplied. For every set of samples being analyzed, a standard curve has to be created.

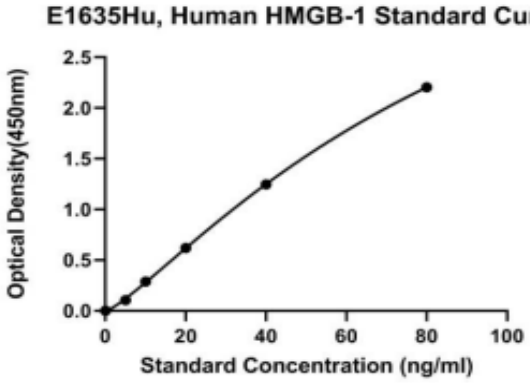


Figure (3-4) standard curve of human HMGB-1

3.3 Statistical Analysis

Statistical analysis was performed with IBM SPSS statistics version 23. Descriptive statistics was performed with the men being summarized using means ± SD. For the estimation of statistical significance, a level of ($p < 0.05$) has been set. The data were determined using the Shapiro- Wilk test to be normal and with Levene's test to be of variances. Correlations between numeric and categorical data were tested using chi-square and Pearson's correlation analyses, respectively. The independent T-test and Mann-Whitney U-test were applied as appropriate to compare two independent groups. The Scheffé post-hoc test for multiple comparisons after ANOVA was used for comparisons of >2 groups, with significance at ($p <0.05$). Receiver Operating Characteristic (ROC) curve analysis was performed to determine cut-off values of individual biomarkers. Aste-risks denote statistically significant differences ($p < 0.05$). Graphical representations were made with GraphPad Prism version 9.

Chapter four

Results

and

Discussions

4.1 The Biomarker in Patients and health.

The table below shows that there is a significant difference observed in the levels of Tetranectine, Protein S100B, and HMGB1 between the control group and both primary and secondary patient groups. Notably, the secondary patient group displayed extreme elevations in Tetranectine levels, indicating the importance of biomarkers in differentiating between disease types. The p-values (< 0.001 in all cases) indicate strong statistical significance for all parameters.

Table 4-1: Evaluation of each patient's research parameters in relation to the control group

Parameters	Control		Patient primary		Patient secondary		P. value
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
Tetranectine (ng/ml)	81.357	12.200	91.869	14.596	3791.574	20166.675	0.00007
Protein S100B (ng/L)	43.161	9.267	50.537	2.711	64.206	9.534	0.00032
HMGB1 (ng/ml)	21.084	2.905	24.738	6.477	37.141	9.331	0.00001

4.2.1 tetranectaine in epileptic patients and health

According to the results, the control group's serum tetranectin level was (81.357 ± 12.200), while the primary group showed (91.869 ± 14.596), and the secondary group demonstrated a markedly elevated level of (3791.574 ± 20166.675). The P-value (< 0.001), indicates a highly significant difference among the groups, also, the tetranectain level between group 1 and 2 of patients is statistically significant (91.869 ±14.596) VS (3791.574 ±20166.675); The P-value (< 0.001) which indicates a highly significant difference.

Notably, tetranectin levels were profoundly increased in secondary epilepsy (see table 4-1).

Tetranectin is a C-type lectin protein primarily involved in remodeling tissue, fibrinolysis (breaking down of blood clots), and development of bones. Additionally, it is known to bind plasminogen, which is involved in the regulation of extracellular matrix and inflammation. (Holtet, *et al.* (1997).

Recurrent seizures in epileptic brains can cause inflammation and damage to neurons. Since tetranectin is linked to extracellular tissue remodeling and inflammatory processes, it may rise in reaction to brain injury brought on by seizures. (Vezzani and Friedman (2011). According to study, people with secondary epilepsy had significantly higher tetranectin levels (as shown in table (4-3). This could be a sign of a compensatory or reactive mechanism to ongoing brain damage. Additionally, the frequency or severity of seizures may be impacted by abnormal tetranectin levels that compromise the blood-brain barrier or neuronal recovery processes (Marchi, *et al.* (2012)). As the condition worsened, the study found that plasma tetranectin levels dropped, with drug-refractory epileptic patients showing noticeably lower levels than the control group (Shukla, *et al.* (2017)).

The hypothesis that tetranectin could serve as an epilepsy biomarker that progresses was further supported by the study's findings that patients with drug-refractory epilepsy had considerably lower serum tetranectin levels.

(Zhai, *et al.* (2010)).

In table (4-1) data, the control group shows a mean tetranectin level of (81.357± 12.200), while the secondary patient group shows a sharp elevation (figure 1a). While this specific pattern disagrees with the reduction observed in study (Sharma, *et al.* (2016), it may reflect distinct biological situations (e.g., acute inflammation). However, the significant variation across groups confirms the relevance of tetranectin as a potential biomarker in the progression of epilepsy. (Hu, *et al.* (2022).

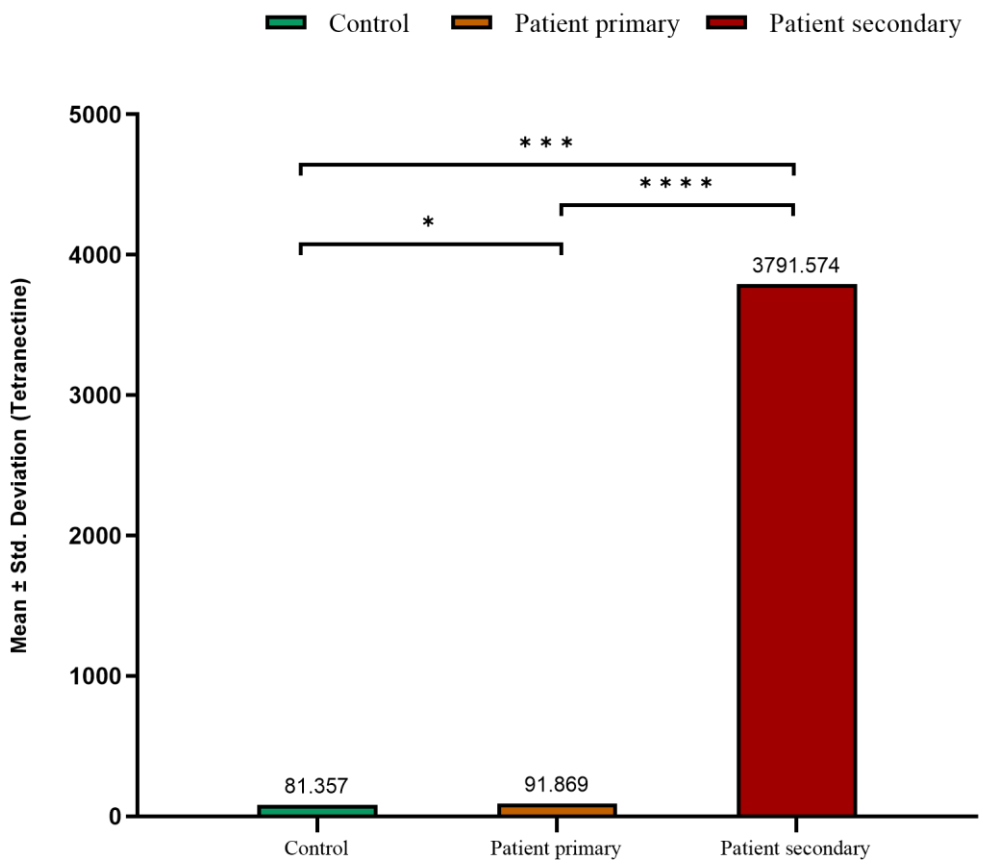


Figure 4.1a: Comparison of the Tetranectin levels of all patients compared with control group.

Tetranectin binds to plasminogen and enhances its activation, aiding in extracellular matrix remodeling and fibrinolysis during wound healing and inflammation which lead to an increase level in serum of patients. (Westergaard, *et al.* (2003). This role in tissue remodeling can lead to elevated tetranectin levels in the serum of patients undergoing active tissue repair or fibrosis. (Doe, *et al*. (2020). Additionally, tetranectin levels increase in certain metabolic disorders, such as type 2 diabetes and others metabolic syndrome, possibly reflecting its involvement in pathological tissue remodeling and altered metabolic signaling. These elevated levels may thus represent a response to acute biological situations where extracellular matrix turnover and cellular remodeling are heightened. (Jiang*, et al.* (2022).

4.2.2 protein S100B in epileptic patients and health

Levels of the protein S100B biomarker in the control group and the other groups (patients in groups 1 and 2) differed statistically significantly; (43.161 ±9.267 VS 50.537 ±2.711, 64.206 ±9.534), P-value (< 0.001). (fig.1b). Also, the comparation between patient groups primary and secondary are statistically significant (50.537 ±2.711VS 64.206 ±9.534); There was a very significant difference between the groups with greater protein S100B levels in the secondary group, as indicated by the P-value (< 0.001). (table 4-1).

Beyond just acting as a marker, S100B plays an active involvement in the pathophysiology of epilepsy. S100B promotes neuronal survival and function at physiological concentrations, but when levels are high, it can cause neurotoxicity by triggering stress-induced enzymes and pro-inflammatory cytokines, which can lead to neuronal apoptosis and worsen epileptic disorders. (Gökçay and Karabiber (2023). Additionally, the interaction of S100B with the receptor for advanced glycation end products (RAGE) has been connected to the development of neuroinflammatory responses, underscoring its dual function of neuroprotection and neurodegeneration contingent on its concentration. (Donato, (2022). An analysis of 18 trials with 1,057 participants revealed that blood S100B levels were considerably greater in epileptic patients than in healthy controls. This lends credence to the idea that elevated S100B levels are linked to epilepsy. (Wang, *et al.,* (2019). Additionally, case-control research on mesial temporal lobe epilepsy (MTLE) revealed that patients had significantly higher plasma S100B levels than healthy controls, which may indicate that raised S100B levels are a biomarker for MTLE." (Lu, *et al.* (2010). Furthermore, serum S100B levels were significantly higher in patients with epileptic seizures (SMD = 0.80; 95% CI 0.18 to 1.42), according to a meta-analysis looking at blood-based brain biomarkers in these patients. This suggests that S100B levels are higher in epileptic patients than in healthy controls. (Kang*, et al.* (2021).

4.2.3 HMGB1 in epileptic patients and health

The HMGB1 levels in the control group (21.084 ± 2.905) were significantly lower compared to those in patients with primary epilepsy (24.738 ± 6.477) and secondary epilepsy (37.141 ± 9.331), with a p-value (< 0.001). A similar significant difference was observed when comparing HMGB1 levels between primary and secondary epilepsy patients (24.738 ± 6.477 vs. 37.141 ± 9.331; ($p < 0.001$), indicating markedly higher HMGB1 levels in the secondary epilepsy group (table 4-1).

HMGB1 plays a crucial role in epilepsy pathogenesis by modulating neuroinflammatory pathways that increase neuronal excitability (Maroso *et al.,* (2021). Targeting HMGB1 and its signaling pathways offer a promising therapeutic strategy, especially for patients unresponsive to conventional treatments (Paudel, *et al.,* (2021).

Elevated HMGB1 levels have been documented in both human epilepsy patients and animal models. For instance, increased HMGB1 expression has been detected in brain tissues of patients with mesial temporal lobe epilepsy and focal cortical dysplasia. Furthermore, serum HMGB1 concentrations are significantly higher in individuals with drug-resistant epilepsy compared to healthy controls (Paudel *et al*., (2021).

A meta-analysis encompassing study reported significantly elevated blood HMGB1 levels in epileptic patients relative to controls, HMGB1 was elevated in both serum and cerebrospinal fluid (CSF), with a more pronounced increase in CSF, underscoring its potential as a reliable biomarker for epilepsy (Chen, *et al.* (2023). Additionally, a 2021 study focusing on drug-resistant epilepsy (DRE) patients found their serum HMGB1 levels significantly exceeded those of healthy individuals, correlating higher HMGB1 levels with more severe epilepsy forms (Zhou *et al.,* 2021).

These findings agree with the current study's data (table 4-1, figure 1b), where HMGB1 mean levels are elevated in both primary and secondary epilepsy groups compared to controls, reinforcing the association between increased HMGB1 and epilepsy..

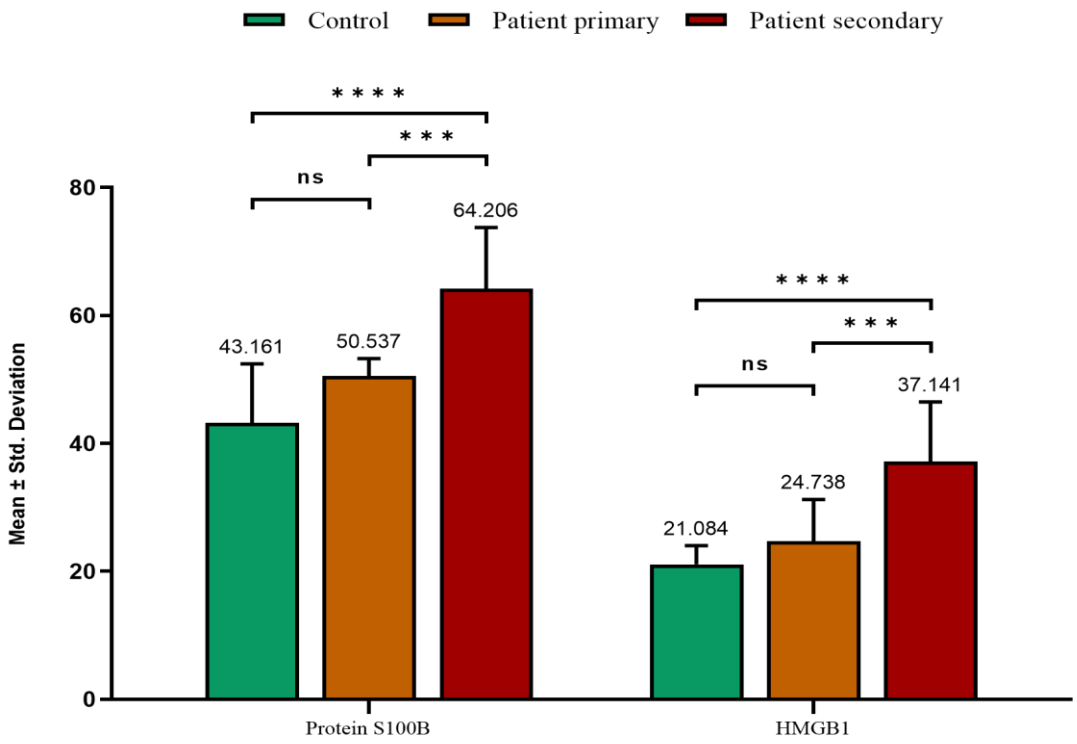


Figure 4.1b: Comparison of the Protein S100B and HMGB levels of all patients compared with control group.

4.3 Comparison of the research parameters of all patients compared with health group based on age group

Table (4-4) compares the levels of three biomarkers—Tetranectine, Protein S100B, and HMGB1—between control subjects and patients at different disease causes, grouped by age: those younger than 30 and those 30 or older. Across all parameters, biomarker levels increase progressively from the control group to the secondary patient group, indicating the effect of age in both epileptic categories.

Table 4-2: Comparison of the research parameters of all patients compared with control group based on age group.

Parameters	Age group	Control		Patient primary		Patient secondary		P. value
		Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
Tetranectine	Less than 30	80.252	14.213	91.243	13.950	104.135	25.017	0.00627
	Grater & equal 30	82.093	11.034	93.589	17.150	6611.381	26788.763	0.00005
Protein S100B	Less than 30	44.526	5.959	50.722	2.870	63.117	7.153	0.00000
	Grater & equal 30	42.251	11.015	50.027	2.312	65.039	11.166	0.00000
HMGB1	Less than 30	21.610	3.814	24.848	7.029	38.445	8.556	0.00004
	Grater & equal 30	20.732	2.157	24.436	5.043	36.145	10.023	0.00002

Tetranectine levels increase slightly in younger patients (from 80.25 ± 14.213) in control groups to (91.243 ± 13.950) in primary group to (104.13± 25.017) in secondary group, with ($p = 0.006$), but in patients aged 30 or older. It sharply increases (from 82.09 ±11.034 in control) to (6611.38 ± 26788.763 in secondary cases). With ($p < 0.001$); This suggests that older individuals may experience a more severe or advanced disease response, at least as reflected by this biomarker. (table 4-2).

This finding agrees with a study published in PubMed that managing epilepsy can be more difficult in elderly people because they frequently have changed metabolic pathways, increased neuroinflammation, and a decreased capacity to respond to treatments. Age-related biomarkers including tau and amyloid-β proteins, together with structural alterations in the brain, have been connected to an increased risk of seizures in older people. (Baram, (2012).

Protein S100B, both age groups show a steady and significant rise in levels from controls to secondary patients. Younger patients go from (44.52±5.959) in control group to (50.72± 2.87) in group-1 to (63.12± 7.15) in group-2, while older patients go from (42.25 ± 11.02) in control group to (50.03 ± 2.31) in group-1 to (65.03 ± 11.17) in group-2, with p-value(= 0.000), indicating a highly significant difference between the groups. (table 4-2). According to the study Serum Protein S100B concentrations have been shown to change with age, with higher levels seen in particular age groups. (Portela, *et al.* (2002). This variation emphasizes how crucial it is to take age into account when assessing S100B as a biomarker for the progression of disease which agrees with figure (4-2).

Similarly, HMGB1 levels increase in both groups, with slightly higher levels in younger secondary patients (38.44±8.56) vs. (36.14± 10.02). All changes are statistically significant ($p < 0.01$), supporting the validity of these differences. (table 4-2).

A study published in Aging Clinical and Experimental Research observed that in healthy adults, serum levels of HMGB1 fall with age, the researchers found that serum HMGB1 levels significantly declined with age in healthy individuals. This reduction suggests that the systemic presence of HMGB1 an important pro-inflammatory molecule diminishes over the course of normal aging, these findings support the idea that the immune and inflammatory response undergoes measurable downregulation as part of physiological aging (Fu, *et al.* (2016). On the other hand, elderly individuals with type 2 diabetes and diabetic kidney disease had considerably higher serum HMGB1 levels, which were correlated with the advancement of the disease. (Liu, *et al*. (2024).

A review also pointed out that age-related features are linked to decreasing intracellular HMGB1 expression and greater extracellular release, suggesting that

HMGB1 may be a universal biomarker for aging and age-related disorders. (Ruggieri, *et al.* (2024).

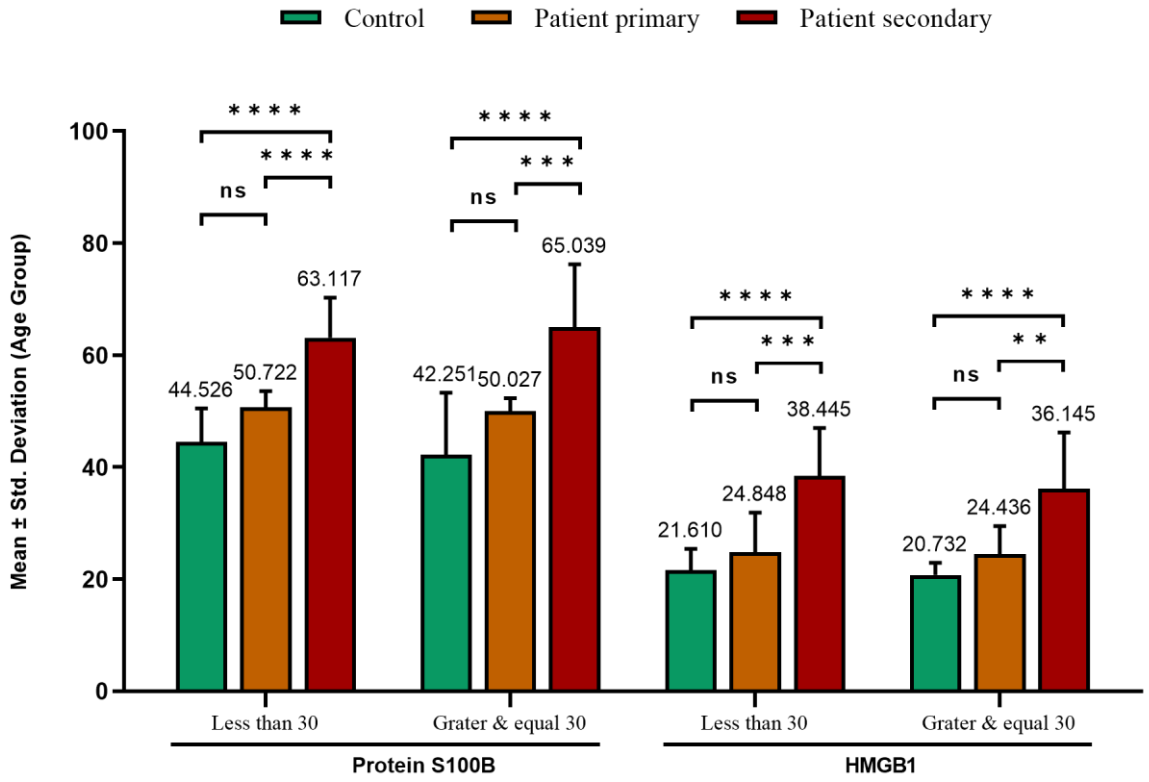


Figure 4-2: Comparison of the research parameters of all patients compared with control group based on age group.

4.4 The Biochemical Parameters of Patients and health Group

4.4.1 Urea and creatinine levels in primary and secondary epilepsy:

The results showed the difference between primary epilepsy, secondary epilepsy and control (25.033 ±4.789g/dl), (25.040 ± 8.590g /dl) vs (25.900 ± 3.458) samples in the level of urea are statistically no significant ($p \geq 0.05$). The same between primary and secondary epilepsy groups (table 4-3).

Table 4-3: Comparison of the research parameters of all patients compared control group

Parameters	Control		Patient primary		Patient secondary		P. value
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
Urea	25.900	3.458	25.033	4.789	25.040	8.590	0.33669
Creatinine	0.613	0.104	0.540	0.072	0.600	0.224	0.01495

A study published in Uremic Encephalopathies: Clinical, Biochemical, and Experimental features discuss the relationship between elevated urea levels and seizures in the context of uremic encephalopathy. The study emphasizes that patients with higher blood urea nitrogen (BUN) levels, especially those with acute kidney injury, may experience severe encephalopathy, including seizures. **(**Daugirdas, *et al.* (2001). According to study, the degree of metabolic abnormalities and renal failure was independently connected with the occurrence of uremic seizures, indicating that high urea levels may be a contributing factor to seizure risk. **(**Kumar and Tripathi (2021)**.** A patient with chronic kidney illness who neglected peritoneal dialysis experienced aphasic status epilepticus, acute uremia aggravation was identified as the cause of the patient's seizure activity, underscoring the link between high urea levels and seizure incidence. **(**Im, *et al.* (2018).

The table (4-3) demonstrated that the three groups differed in terms of the control group's creatinine level; patients and the control group are statistically significant (0.540 ± 0.072mg/dl), (0.600 ± 0.224mg/dl) vs (0.613 ± 0.104mg/dl). Comparing the creatinine levels of individuals with primary and secondary epilepsy revealed the same results. ($p < 0.05$).

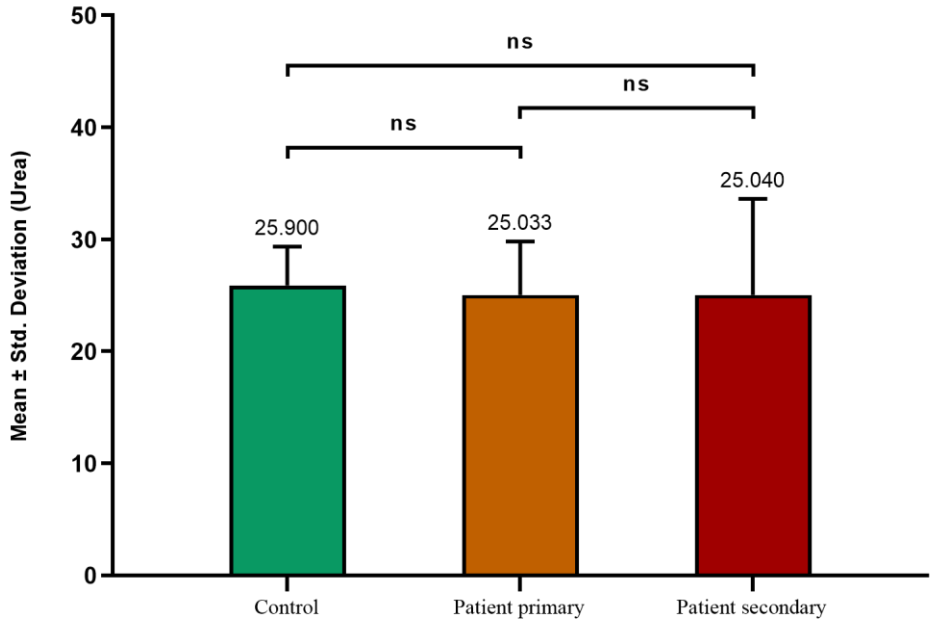


Figure 4.3a: Comparison of the urea levels of all patients compared with control group.

Urea levels are consistent across all groups and show no discernible variation (P=0.33669). The fact that every patient in the research had consistent urea levels might be the cause of this.

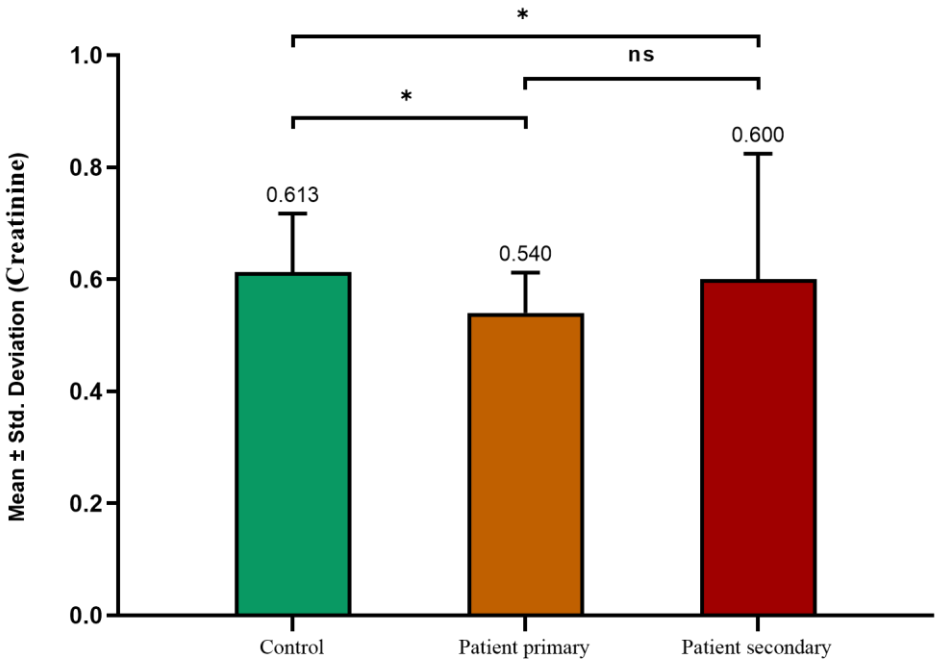


Figure 4.3b: Comparison of the creatinine levels of all patients compared with control group.

Creatinine levels: significant difference ($p < 0.05$) slightly lower in primary epilepsy compared to controls and secondary, this is because the symptoms related to kidney failure are considered secondary epilepsy, not primary epilepsy. A typical indicator of compromised renal function is elevated serum creatinine. The buildup of uremic toxins in acute or chronic renal illness can have an impact on the central nervous system, which may result in a reduction in the seizure threshold. Those who are impacted may be at higher risk of seizures as a result. (Harden, (2021). Therefore, people with CKD may be more susceptible to seizures. When renal failure is present, uremic seizures can manifest as non-convulsive periods and are relatively common. Significantly increased serum creatinine levels, which indicate compromised renal function, are frequently linked to these seizures. (Li, *et al.* (2018). Following a tonic-clonic seizure, a patient experienced acute kidney damage (AKI), the authors pointed out that the patient's renal impairment may have caused the seizure, and the patient's serum creatinine level rose noticeably, this emphasizes the possible connection

between high creatinine levels and the incidence of seizures. **(**Cheng, *et al*. (2014)); which agree with the result in table (4-3). Also, it found that increased serum creatinine levels were associated with a higher risk for developing CKD. The results indicate that elevated creatinine levels may be a sign of underlying renal failure, which may make people more susceptible to seizures. (Hwang and Kim (2020).

4.4.2 Calcium in primary and secondary epilepsy:

The results showed that the control group had a serum calcium level of (8.817 ± 0.420 mg/dl), compared to (8.843 ± 0.603 mg/dl) in the primary group and (7.810 ± 0.934 mg/dl) in the secondary group (P< 0.001), indicating a highly significant difference between the groups with a notable lower calcium level in the secondary group. (table 4-4).

Table 4-4: Comparison of the research parameters of all patients compared with control group

Parameters	Control		Patient primary		Patient secondary		P. value
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
BMI	22.822	2.346	22.965	3.682	23.045	2.789	0.51522
Ca++	8.817	0.420	8.843	0.603	7.810	0.934	0.00004
HBA1C	6.613	0.580	6.877	0.564	5.320	1.851	0.00603

while the difference between the primary epileptic group and secondary epileptic group was also significant (8.843 ±0.603 mg/dl) VS (7.810 ±0.934 mg/dl), (P < 0.001) which indicates a highly significant difference. (table 4-4).

The individuals with epilepsy had significantly lower blood calcium levels than healthy controls. According to the study, there was a statistically significant difference ($p < 0.05$) between the mean serum calcium levels of the control

group and the patients with epilepsy, **(**Reddy and Kuruba, (2007). The study found that calcium levels were significantly low**er** in the seizure group compared to healthy controls, with statistical significance ($p < 0.05$). These findings suggest that electrolyte imbalances, particularly hypocalcemia, may contribute to seizure susceptibility or severity in adults (Subbalakshmi, *et al.* (2007).

4.4.3 HBA1C in primary and secondary epilepsy:

The results demonstrated a significant difference in HbA1c levels among patients with primary epilepsy, secondary epilepsy, and the control group, with values of (6.877 ± 0.564) mg/dL, (5.320 ± 1.851) mg/dL, and (6.613 ± 0.580) mg/dL, in turn. A statistically significant difference, especially between the primary and secondary epilepsy groups, is shown by the p-value of 0.006 (table 4-4).

Further comparison between the primary and secondary epilepsy groups showed that the primary group had a higher mean HbA1c level compared to the secondary group with this difference being statistically significant ($p< 0.05$).

These results support the link between elevated HbA1c levels and increased seizure risk. A study found that patients with poor glycemic control had a significantly higher risk of generalized seizures (Xu *et al.,* 2017). Additionally, a 2020 study in Epilepsy found a correlation between elevated HbA1c levels and occipital lobe seizures, further reinforcing the link between high HbA1c and seizure activity (Fujimoto *et al.,* 2020).

4.4.4 BMI in patient and health groups:

The BMI level was similar across all groups: (22.82 ± 2.346) in the control group, (22.965±3.682) in the primary patient group, and (23.05 ±2.789) in secondary patient group. (P = 0.51522) indicates no statistically significant difference in BMI between the groups. (table 4-4). The difference between two group of patients was also not significant.

The relationship between body mass index (BMI) and epilepsy progression and treatment is becoming more widely acknowledged. According to studies, people with greater BMI may have more seizures, perhaps as a result of changes in the brain caused by inflammation and the altered pharmacokinetics of antiepileptic medications in adipose tissue. (Janousek, *et al.* (2020). Additionally, obesity is linked to disorders like sleep apnea, which can worsen seizure activity by interfering with oxygen delivery and sleep cycles. (Vaughn, *et al*. (2018).

On the other hand, underweight people may experience dietary shortages that affect neuronal function and lessen the efficacy of seizure control treatments. (Kim, *et al.* (2017).

Furthermore, BMI can affect the likelihood of adverse drug reactions; obese patients are more likely to experience metabolic problems when taking specific antiepileptic drugs. (Perucca, (2019).

4.5 Correlation Coefficient Among Parameters According to Research Parameters

The correlation analysis presented in table (4-10) explains the relationships between various biological and clinical parameters in epilepsy patients. A notable finding is the positive correlation between Tetranectine and both Protein S100B (r = 0.318, p = 0.013) and HMGB1 (r = 0.404, p = 0.001). These statistically

significant results suggest that as Tetranectine levels rise, the levels of S100B and HMGB1 rise, which are both established markers of neuronal damage and neuroinflammation.

Protein S100B also shows a strong positive correlation with HMGB1 ($r = 0.537$, $p < 0.001$), confirming the idea that these proteins might cooperate in reaction to chronic inflammation or brain damage. Interestingly, S100B is negatively correlated with calcium levels ($r = -0.439$, $p < 0.001$) and HBA1C ($r = -0.302$, $p = 0.019$), suggesting a potential link between S100B and altered metabolic or mineral states in epilepsy patients.

S100B and Calcium Levels: A study that was published in Frontiers in Neurology looked at the connection between calcium homeostasis and S100B levels in individuals with epilepsy. A possible connection between S100B and disturbed calcium homeostasis in epilepsy was suggested by the study's findings that S100B levels were considerably higher in epileptic patients than in controls and that these levels had an inverse relationship with blood calcium levels. (Liang, *et al.* (2019).

S100B and HbA1c Levels: A study published in Diabetes Research and Clinical Practice investigated the relationship between glycemic control and S100B levels in diabetic patients. Higher S100B levels were linked to better glycemic control, according to the study, which also identified a negative correlation between S100B levels and HbA1c levels. This study indicates a possible association between S100B and metabolic parameters that may be pertinent to individuals with epilepsy, even though it was carried out on diabetic patients. (Katsanou, *et al.* (2018).

HMGB1 displays a significant negative correlation with calcium ($r = -0.382$, $p = 0.003$), which could reflect an imbalance in neuronal excitability or ionic regulation in the brain. However, HMGB1 did not significantly correlate with

seizure duration, indicating that while it reflects injury, it may not linearly track disease length.

Table 4-5: Correlation Coefficient Among Parameters According to Research Parameters

Parameters	Value	Protein S100B	HMGB 1	Ca++	HBA1C	Urea	Creatinine	Age	BMI	Seizure duration
Tetranectine	R. value	.318*	.404**	-.215	-.173	-.283*	-.074	.189	.035	.190
	P. value	.013	.001	.099	.185	.028	.575	.148	.790	.145
Protein S100B	R. value	1.000	.537**	-.439**	-.302*	-.014	-.006	.205	-.051	.108
	P. value		.000	.000	.019	.913	.965	.117	.700	.412
HMGB1	R. value		1.000	-.382**	-.012	.073	.097	.141	-.195	.023
	P. value			.003	.926	.577	.460	.282	.135	.862
Ca++	R. value			1.000	-.210	.223	.211	-.201	.151	-.127
	P. value				.107	.087	.106	.124	.251	.334
HBA1C	R. value				1.000	-.127	-.303*	-.072	.043	-.011
	P. value					.334	.019	.585	.742	.931
Urea	R. value					1.000	.413**	-.072	-.175	.109
	P. value						.001	.582	.181	.405
Creatinine	R. value						1.000	-.040	-.119	.066
	P. value							.761	.364	.614
Age	R. value							1.000	.593**	.471**
	P. value								.000	.000
BMI	R. value								1.000	.299*
	P. value									.020

High mobility group box 1 (HMGB1) protein has been shown to influence and be influenced by intracellular calcium (Ca^{2+}) signaling, establishing a bidirectional relationship important in various physiological and pathological processes. (Yang, *et al.* (2013). Calcium-dependent processes can cause HMGB1 release from cells, especially in reaction to stress or injury, where Ca^{2+} influx promotes HMGB1 translocation from the nucleus to the cytoplasm and following secretion. (Bonaldi, *et al.* (2003). Once extracellular, HMGB1 can interact with cell surface receptors such as RAGE and TLR4, which in turn trigger signaling cascades that alter the dynamics of intracellular Ca^{2+}. (Tang, *et al.* (2007). Furthermore, via encouraging calcium overload and mitochondrial dysfunction, prolonged HMGB1 signaling has been linked to calcium dysregulation, especially in inflammatory and neurodegenerative disorders. (Scaffidi, *et al.* (2002).

Seizure duration itself shows strong correlations with age ($r = 0.471$, $p < 0.001$) and BMI ($r = 0.299$, $p = 0.020$), which is expected—older and heavier patients are likely to have had epilepsy for a longer period. A study published at 2016 suggests that longer duration of epilepsy and aging are key contributors to the development of a range of comorbidities, including cardiovascular disease, metabolic syndrome, and obesity, as patients with chronic epilepsy often experience cumulative side effects of antiepileptic drugs (AEDs), lifestyle limitations, and reduced physical activity—all of which can lead to higher BMI and worsened metabolic health. (Keezer, *et al.* (2016), which agrees with the original finding that seizure duration shows correlations with both age and BMI, due to long-term disease progression and associated health effects. Tetranectine, S100B, and HMGB1 also showed positive but non-significant correlations with seizure duration, suggesting a possible pattern that would become more apparent with longer-term monitoring or a larger sample size.

4.6 Prediction incidence of disease

4.6.a prediction of subject working characteristics based on the health and patients.

Table (4-6) presents the model prediction of subject working characteristic curves for three biomarkers—Tetranectine, Protein S100B, and HMGB1—based on data from control and patient groups. The results show that the best diagnostic performance was shown by Protein S100B, which had a moderate specificity (70.00%), very high sensitivity (98.33%), and an area under the curve (AUC) of 86.36%, demonstrating a high capacity to accurately detect positive cases. HMGB1 also showed high performance, with an **AUC of** 82.78%, and a more balanced diagnostic profile: sensitivity of 70.00% and specificity of 90.00%, making it useful for both ruling in and ruling out disease. Tetranectine, while having the lowest AUC at 79.08%, still demonstrated acceptable sensitivity (70.33%) and specificity (80.00%). All three markers had statistically significant levels ($p < 0.01$) and reasonably narrow confidence ranges, indicating strong model predictions. According to these results, Protein S100B might be a particularly useful diagnostic biomarker. Tetranectine and HMGB1 also show potential, particularly when considered together for therapeutic applications.

Table 4-6: Model prediction of subject working characteristic curves according to research parameters based on the control and patients.

Metrics		Tetranectine	Protein S100B	HMGB1
Std. Error		0.048	0.041	0.042
Asymptotic Sig.		0.007	0.002	0.004
Asymptotic 95% Confidence Interval	Lower Bound	0.696	0.784	0.745
	Upper Bound	0.885	0.943	0.911
Cutoff Point		88.973	45.165	23.994
Area Under Curve (AUC)		79.083%	86.361%	82.778%
Sensitivity		70.333%	98.333%	70.000%
Specificity		80.000%	70.000%	90.000%

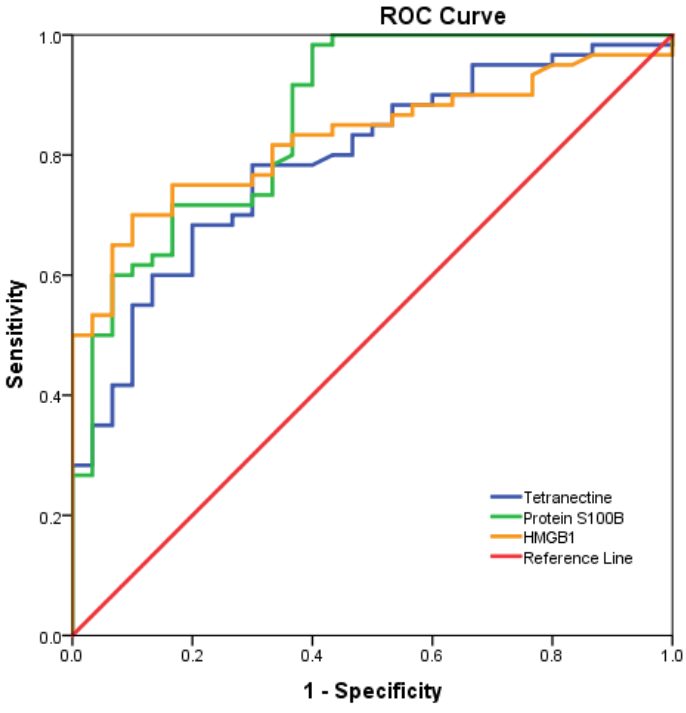


Figure 4.4: Model prediction of subject working characteristic curves according to research parameters based on the Control and patients.

The Receiver Operating Characteristic Curve, or ROC curve, is displayed in figure (4-6) to demonstrate how well the biomarkers work as diagnostic tools for differentiating between patient and control groups. The real positive rate (sensitivity) is displayed on the y-axis, whilst the false positive rate (1 - specificity) is represented on the x-axis. The green line that represents Protein S100B is the one that reaches the upper-left corner of the three curves, suggesting that it provides the highest sensitivity and specificity across thresholds. In comparison, the blue (Tetranectin) and orange (HMGB1) lines perform well but fall slightly below the green curve, suggesting good but comparatively lower diagnostic accuracy. The red diagonal reference line, which reflects random guessing (AUC = 0.5), serves as a baseline for interpretation. According to a visual estimation it suggests that Protein S100B has the highest AUC, followed by Tetranectin and HMGB1. The steep early rise of the green curve also supports Protein S100B's excellent ability to detect patients with minimal false positives. Overall, this analysis indicates that Protein S100B is the most accurate biomarker among the three for differentiating between patients and controls in this study.

A basic graphical technique that is frequently used in biomedical research to evaluate the diagnostic accuracy of tests and biomarkers is the Receiver Operating Characteristic (ROC) curve. Plotting the genuine positive rate (sensitivity) versus the false positive rate (1 − specificity) at several threshold levels helps determine the ideal cutoff value (Hajian-Tilaki, 2013). The Area Under the Curve (AUC), which measures the test's overall capacity to distinguish between positive and negative instances, is a crucial metric that is obtained from the ROC curve. Perfect diagnostic accuracy is represented by an AUC of 1.0, whereas no discriminative capability is shown by an AUC of 0.5. ROC analysis is especially valuable for comparing multiple biomarkers or diagnostic models,

thereby assisting clinicians and researchers in selecting the most effective diagnostic tools (Liu *et al.,* 2023)

4.6.b prediction of subject working characteristics based on the primary and secondary patients.

Table (4-7) presents the model prediction of subject working characteristic curves of three biomarkers—Tetranectin, Protein S100B, and HMGB1—based on predictions for primary and secondary patient groups.

Table 4-7: Model prediction of subject working characteristic curves according to the research parameters based on the primary and secondary patients.

Metrics		Tetranectine	Protein S100B	HMGB1
Std. Error		0.064	0.023	0.046
Asymptotic Sig.		0.001	0.005	0.001
Asymptotic 95% Confidence Interval	Lower Bound	0.619	0.922	0.775
	Upper Bound	0.870	1.000	0.954
Cutoff Point		96.394	54.559	25.698
Area Under Curve (AUC)		74.444%	96.667%	86.444%
Sensitivity		73.333%	90.000%	90.000%
Specificity		70.667%	96.667%	70.000%

Protein S100B shows the highest diagnostic accuracy, with an AUC of 96.667%, showing excellent distinguishing ability. It also has the highest specificity (96.667%) and strong sensitivity (90.000%), confirming its status as the most reliable marker among the three. HMGB1 also demonstrates high sensitivity (90.000%) and a respectable AUC of 86.444%, though its specificity is lower (70.000%). Tetranectin, with an AUC of 74.444%, sensitivity of 73.333%, and specificity of 70.667%, lowest in diagnostic performance, all

three markers showing significance ($p < 0.01$). Overall, Protein S100B emerges as the best biomarker for distinguishing between control and patient groups, supported by both the curve characteristics and the quantitative metrics.

A study performed a Receiver Operating Characteristic (ROC) curve analysis, demonstrating a remarkble AUC of 0.980, signifying excellent diagnostic accuracy. Moreover, this study showed a significant correlation between S100B levels and seizure severity as well as MRI abnormalities, indicating that this biomarker not only aids in early diagnosis but may also reflect underlying brain pathology (El-Naggar, *et al*. (2023).

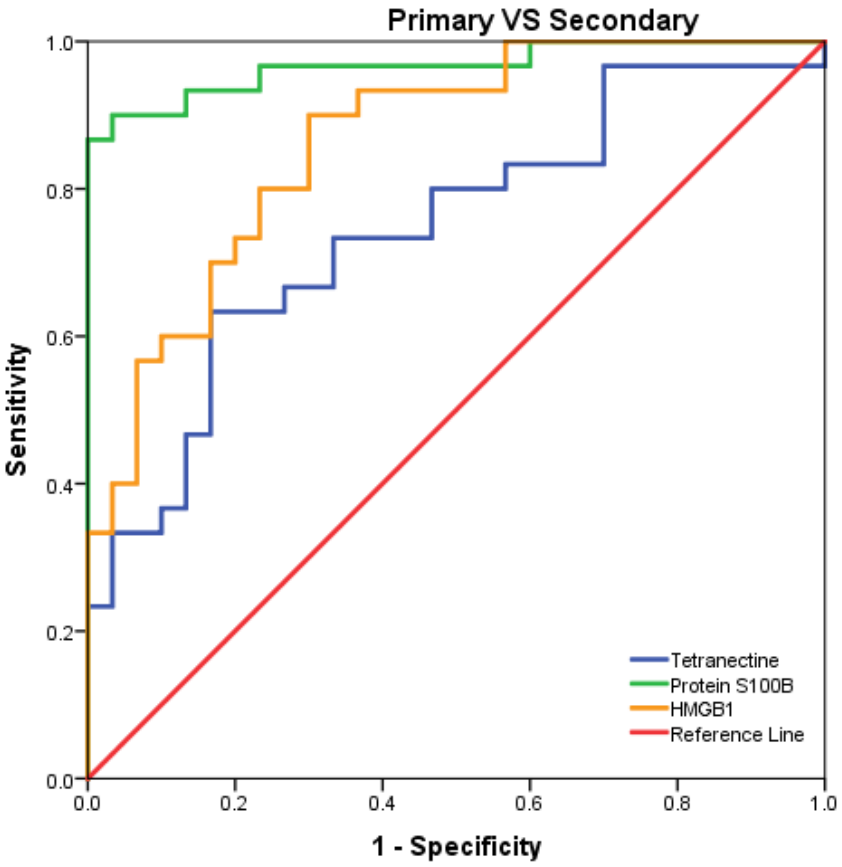


Figure 4.5: Model prediction of subject working characteristic curves according to research parameters based on the primary and secondary patients.

Conclusions

and

Recommendations

Conclusion and Recommendations

Conclusions

The Current study concludes the following:

1. The results conclude that the levels of Tetranectine, Protein S100B and HMGB1 are used to differentiate between patients with epilepsy and healthy people as well as to differentiate wither patients have seizure due to genetic predisposition or due to acquired causes.
2. The results indicate a decrease in HbA1c and calcium levels in patients with secondary epilepsy.
3. The findings reveal an increase in creatinine levels among secondary epileptic patients.
4. A positive correlation was observed between tetranectin and both proteins S100B and HMGB1, as well as between proteins S100B and HMGB1. Additionally, seizure duration showed a positive correlation with both age and BMI.
5. There is a positive relationship between the incidence of primary and secondary epilepsy with age.
6. There is a negative reverse relationship of S100B with HBA1C and calcium. As well as the negative correlation between HMGB1 and calcium level.
7. The correlational relationships between other parameters in study are found but they were statistically non-significant
8. The results conclude that protein S100B could be used to predicate the possibility of having epilepsy disease.

Conclusion and Recommendations

Recommendations

1. Studying the genetic cause of epilepsy that are caused by *SCN1A* mutations.
2. Studying the autoimmune diseases that lead to epilepsy, such as seizures that occur in lupus patients.
3. Studying the comparation between the effects of calcium, HBA1C, urea and creatinine in patients with secondary epilepsy.
4. It is recommended to confirm the cause of the disease to avoid incorrect treatments.
5. It is recommended to follow an appropriate diet for people suffering from seizures due to low calcium, low blood sugar, and kidney dysfunction.
6. An in-depth study of epilepsy that occurs during sleep (Nocturnal seizure).
7. An in-depth study of treatments used to treat epilepsy and their long-term effects.
8. Studying of the causes of epilepsy caused by psychological and academic stress.

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Appendices

Appendix 1

Questioner for participants

Form number: ……..

Patient name: ……..

Patient age: ……..

Patient weight: ……..

Patient height: ……..

the question yes or no Additions

Do you suffer from chronic diseases:

Does the patient suffer from a brain injury, stroke, or any disease of the nervous system?

Do you suffer from epilepsy?

Date of onset of illness?

seizure period?

seizure time? Morning / evening/ during sleep/ not specific time

The period between one seizure and another? ……

Do you take medication? Yes/No

Are you responding to treatment? Yes/ No

مجموعات الاصحاء والمرضى في المجموعة الأولية والثانوية، ولكن في المرضى الذين تبلغ أعمارهم 30 عامًا أو أكثر، فإنها تزداد بشكل حاد حتى تبلغ اعلى مستوى في المجموعة الثانوية وهذا يشير إلى أن الأفراد الأكبر سناً قد يعانون من استجابة مرضية أكثر شدة، كما يظهر بروتين S100B في كلتا الفئتين العمريتين ارتفاعًا كبيرًا في المستويات من الاصحاء إلى المرضى الثانويين مما يشير إلى فرق كبير الدلالة بين المجموعتين. وبالمثل، ارتفعت مستويات HMGB1 في كلتا المجموعتين، مع ارتفاع طفيف في المرضى الأصغر سنًا مقابل المرضى الأكبر سنًا. جميع التغييرات ذات دلالة إحصائية (قيمة الاحتمالية <0.01).

أظهرت نتائج الدراسة وجود فرق معنوي في مستوى الكرياتينين (P<0.01) - أقل قليلاً في الصرع الأولي مقارنة بالاصحاء والصرع الثانوي، فضلاً عن وجود فرق معنوي كبير للغاية (p<0.000) في مستويات الكالسيوم في المرضى الذين يعانون من الصرع الأولي مقارنة بالأفراد الأصحاء مع انخفاض ملحوظ في مستوى الكالسيوم في المجموعة الثانوية. وأظهرت النتائج انخفاضًا ملحوظًا في مستويات HBA1C لدى المرضى الذين يعانون من الصرع الثانوي مقارنة بمجموعة الاصحاء ومرضى الصرع الأولي.

كانت هناك علاقات ارتباطية بين المعايير المستخدمة في البحث، مثل الارتباط الإيجابي بين ال Tetranectin وكل من البروتين S100B وHMGB1 . كما يظهر البروتين S100B أيضًا ارتباطًا إيجابيًا قويًا مع HMGB1 ويرتبط سلبًا بمستويات الكالسيوم و السكر التراكمي بالإضافة إلى ارتباط سلبي كبير بين HMGB1 والكالسيوم.

تُظهر مدة النوبة نفسها ارتباطات قوية بالعمر ومؤشر كتلة الجسم. أظهرت الTetranectin ، وS100B، وHMGB1 أيضًا ارتباطات إيجابية، وإن كانت غير دالة إحصائيًا مع مدة النوبات. وركزت الدراسة أيضًا على إمكانية التنبؤ بمرض الصرع، سواءً كان له سبب وراثي أو مكتسب، وأظهرت النتائج أن أفضل أداء تشخيصي كان من نصيب بروتين S100B، الذي اظهر قدرة عالية على الكشف الدقيق عن الحالات الإيجابية. كما أظهر بروتين HMGB1 أداءً عاليًا ونمطا تشخيصيا أكثر توازنًا. أما الTetranectin ، فرغم أنه سجّل أدنى نسبة مساحة تحت المنحنى (AUC)، إلا أنه أظهر حساسية وخصوصية مقبولتين.

وفقًا للنتائج المذكوره أعلاه، يمكن استخدام هذه المؤشرات الحيوية: الTetranectin ، والبروتين S100B، وHMGB1 للتمييز بين مرضى الصرع سواء الأولي أو الثانوي والتنبؤ بإمكانية الإصابة بهذا المرض.

الخلاصة

الصرع مرض دماغي، يتميز بميل مستمر للإصابة بنوبات، وتُعدّ الآثار العصبية الحيوية والمعرفية والنفسية والاجتماعية لتكرار النوبات من السمات المميزة للصرع. تُعرّف النوبات الانتيابية المتكررة، أو نوبات الصرع، باضطرابات سلوكية نمطية تُشير إلى الآليات الدماغية الكامنة وراء المرض. هناك العديد من الاضطرابات السريرية التي يُدرجها التشخيص التفريقي للصرع، وتتميز بتغيرات وجيزة في الوعي و/أو السلوك. ويُصنف الصرع حسب سبب المرض إلى صرع أولي يشمل السبب الوراثي، وصرع ثانوي يشمل السبب المكتسب لهذا المرض.

هدفت هذه الدراسة إلى التمييز بين مرضى الصرع والأصحاء، وكذلك التمييز بين المرضى الذين يعانون من نوبات بسبب الاستعداد الوراثي (الصرع الأولي) أو نتيجة لأسباب مكتسبة (الصرع الثانوي) من خلال قياس مستويات المؤشرات الحيوية للTetranectin ، والبروتين S100B، وHMGB1، والتي يُمكن استخدامها أيضًا للتنبؤ باحتمالية الإصابة بمرض الصرع. أُجريت الدراسة في مختبرات مستشفى الكفيل ووحدة تخطيط كهربية الدماغ في مستشفى الحسين التعليمي بكربلاء، خلال الفترة من نوفمبر 2024 إلى أبريل 2025. شارك في الدراسة 90 شخصًا، تراوحت أعمارهم بين 15 و70 عامًا. ثلاثون منهم أصحاء، وثلاثون مصابًا بالصرع الأولي، وثلاثون مصابًا بالصرع الثانوي (مرضى).

وفقًا لنتائج هذه الدراسة، لوحظ وجود فرق كبير في مستويات الTetranectin بين مجموعة الاصحاء ومجموعتي المرضى الأوليين والثانويين، كما أن مستوى الTetranectin بين المجموعة الأولية والثانوية من المرضى ذو دلالة إحصائية حيث ارتفع بشكل كبير للغاية في المجموعة الثانوية. كانت هناك فروق ذات دلالة إحصائية في مستوى البروتين S100B بين مجموعة الاصحاء والمرضى الذين يعانون من الصرع الأولي والثانوي، كما أن المقارنة بين مجموعتي المرضى الأوليين والثانويين ذات دلالة إحصائية مع ارتفاع مستوى البروتين S100B في المجموعة الثانوية.

وفيما يتعلق بمستوى HMGB1 في مجموعة الاصحاء أظهرت النتائج فرقًا إحصائيًا كبيرًا ($p<0.000$) عند مقارنتها بمرضى الصرع الأولي والصرع الثانوي، وكان الأمر نفسه صحيحًا عند مقارنة مستوى HMGB1 في مرضى الصرع الأولي الصرع الثانوي مما يشير إلى وجود فرق كبير جدًا بين المجموعات وكان مستوى HMGB1 الأعلى في المجموعة الثانوية.

جامعة كربلاء

كلية العلوم الطبية التطبيقية

المقارنة بين مستويات ال Tetranectin, Protein S100B و High-Mobility Group Box-1 Protein لدى مرضى الصرع بنوعيه الأولي والثانوي

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية _جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في علوم التحليلات المرضية

من قبل

عذراء زين العابدين موسى

بكالوريوس تحليلات مرضية

كلية العلوم الطبية التطبيقية/جامعة كربلاء, 2022

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