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Clinical Significance of Glutamate Dehydrogenase 2, Sirtuin 4, some Elements in Epileptic Patients

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***Abstract*–Epilepsy is a neurological condition caused by an imbalance of excitation and inhibition in the brain. Many factors influence this imbalance, including enzymes involved in producing neurotransmitters, the balance of electrolytes, and the effect of trace elements. The importance of this research lies in assessing the levels of these enzymes in human blood serum, unlike the common research specially in Kurdistan Thus, this study aimed to investigate the levels of glutamate dehydrogenase 2 (GDH2), NAD-dependent ADP-ribosyl transferase sirtuin 4 (SIRT4), electrolyte (Na, K, and Cl), and trace elements (Zn, Cu, and Se) in epileptic patients and compare them with control groups. The blood serum from 30 focal epilepsy (FE), generalized epilepsy (GE) patients, and 30 control groups was collected in Erbil city Hospital. The results of the study showed a significant decrease in GDH2 with a $p<0.0001$ for the FE and GE groups. SIRT4 levels were significantly increased in both groups compared to the control group. Furthermore, levels of Na, Zn, and Se were markedly reduced, while no significant variations were observed in K, Cl, and Cu levels between epileptic patients and control groups. The receiver operating characteristic curve and area under the curve results for GDH2 (0.8092), SIRT4 (0.8956), sodium (0.8673), zinc (0.9711), and selenium (1). The results of the artificial neural networks showed that selenium, zinc, sodium, GDH2, and SIRT4 had the greatest impact on how the network classified patients as having epilepsy or being healthy. The results of GDH2, SIRT4, Na, Zn, and Se may be utilized as parameters for detecting epileptic patients.**

***Index Terms*—Electrolytes, Epilepsy, Glutamate dehydrogenase 2, NAD-dependent ADP-ribosyltransferase sirtuin 4, Trace elements.**

I. Introduction

Epilepsy is a serious neurological disorder ranked among the top three neurological diseases globally (Huang, et al., 2023). The condition is characterized by unprovoked, recurrent seizures resulting from excessive neuronal firing and transient central nervous system dysfunction (Domańska, et al., 2025). The pathophysiology involves an imbalance in electrical stimulation, primarily between the excitatory neurotransmitter glutamate and the inhibitory gamma-aminobutyric acid (GABA). Hyperexcitability occurs through elevated glutamate levels or reduced GABA-mediated inhibition (Gruenbaum, et al., 2024). The accumulation of toxic metabolic products causes excessive neuronal discharge, which in turn causes a cascade of metabolic imbalances, including defects in enzymatic biochemical pathways (Shi, et al., 2024). Several factors contribute to seizure activity, including neuronal cell death, programmed cell death, oxidative stress, mitochondrial dysfunction, and membrane potential instability. Epileptic seizures increase neuronal energy demands and trigger these conditions (Ruhling, Hartmann and Das, 2024).

Glutamate dehydrogenase (GDH, EC: 1.4.1.3) catalyzes the reversible reaction of L-glutamate using NAD $(P)^+$ as cofactors to α-ketoglutarate and ammonia; it's a mitochondrial matrix enzyme (Kravos, 2021). Acting as a key regulator for amino acid and ammonia metabolism in the human pancreas, liver, and brain, it is an essential enzyme that connects carbon and nitrogen metabolism while contributing to energy homeostasis (Zeng, et al., 2025). There are two GDH isoenzymes with distinct properties and tissue distribution in humans. GDH 1 (GDH1), encoded by the *GLUD1* gene, is expressed in all human tissues with the highest levels in the liver, while GDH 2 (GDH2), encoded by the *GLUD2* gene, shows predominant expression in the brain, kidney, testis, and steroidogenic organs (Litso, et al., 2023). Dysregulation of GDH activity significantly impacts neurological health, with deficiency or overexpression contributing to disorders including Parkinson's disease, epilepsy, Alzheimer's disease, and spinocerebellar atrophy through effects on energy metabolism and glutamate homeostasis. GDH2 is considered an important mediator for TCA cycle regeneration through glutamate, as GLUD2 expression in astrocytes enhances glutamate uptake and oxidative metabolic capacity, especially during increased workload and elevated blood glucose (Drews, et al., 2020).

Sirtuins (SIRT) are a family of NAD^+-dependent deacetylases and ADP-ribosyltransferases consisting of seven

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isoforms (SIRT1-7). SIRT4 functions as a multifunctional enzyme, catalyzing lipoamidase, ADP-ribosyltransferase, and deacetylase activities. It is a mitochondrial matrix protein (Xu, et al., 2023). SIRT4 maintains mitochondrial homeostasis by regulating proteins involved in metabolic reactions, autophagy, and antioxidant pathways (Wu, et al., 2022). SIRT4 inhibits GDH activity and limits glutamate and glutamine metabolism by regulating gluconeogenesis and glutamate metabolism, specifically by catalyzing the transfer of ADP-ribose from NAD^+ to GDH (Tomaselli, et al., 2020). This enzyme is involved in regulating cellular metabolism by controlling NAD^+ metabolism and the NAD^+/NADH ratio, particularly under toxic stress conditions (Han, et al., 2019). SIRT4 affects the GABA/glutamate ratio that determines neuronal excitability by regulating GDH activity. The enzyme is involved in the growth of astrocytes, regulates the growth of glial cells, and restores mitochondrial function. Thus, it protects the brain during its growth and function on neural tissue, indicating its potential role in maintaining the balance of glutamate and GABA to control epilepsy (Qiao, et al., 2024).

Electrolyte hemostasis is fundamental to proper brain function, involving complex regulatory mechanisms including the blood–brain barrier, neuronal and glial membrane properties, and intricate molecular systems. Changes in electrolyte levels across the cell membrane directly and indirectly affect neuronal discharges and promote epileptic activity; therefore, a delicate ionic balance is essential in the brain (Kamlesh, Singh and Srivastava, 2022). When electrolyte levels, such as sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), and magnesium (Mg^{2+}), are disrupted, various neurological symptoms appear, ranging from neuromuscular abnormalities to serious complications (Netravathi, Ashrit and Patil, 2025). Cerebral edema, seizure, and encephalopathy are all conditions caused by severe hyponatremia (low serum sodium levels). Meanwhile, potassium imbalances affect both cardiac and neuromuscular functions (Howard, et al., 2025).

The elements such as zinc (Zn), copper (Cu), and selenium (Se), known as trace elements, are fundamental in building the central nervous system. Act as enzyme cofactors in various metabolic processes. Change in these factors impairs antioxidant defense systems, which leads to neuronal excitotoxicity and more seizures (Kakkar, et al., 2020). Zn has many neuroprotective effects. Act a cofactor for glutamic acid decarboxylase, it changes how easily neurons fire, and it is the main part of superoxide dismutase (SOD). This antioxidant enzyme cleans up oxidative radicals that damage neuronal membranes and cause epilepsy (Tavasoli, Afsharkhas and Parvini, 2024). Copper is necessary for the formation and functioning of the nervous system and for some enzymes in the brain. It is necessary for making neurotransmitters and for the growth of the central nervous system. It works with redox enzymes such as cytochrome oxidase and SOD to improve cellular respiration and protect cells from oxidative stress (Fan, et al., 2024). Se plays a main role through selenoproteins in neurotransmission and neuroprotection. These proteins are necessary for dopaminergic and GABAergic neurotransmission. This has an effect on both cognitive and motor skills. Selenoproteins protect neurons from inflammation, oxidative damage, and cell death, and act as survival factors for them (Al Omairi, et al., 2022).

Even though diagnosing and treating epilepsy is becoming more advanced, exactly what biochemical pathways start and drive seizures is still unknown. Previous studies have rarely established systematic associations between these biochemical changes and clinical responses to different epilepsy types or therapeutic strategies. Given the limited research on epilepsy in Kurdistan and the fact that most existing studies have focused on laboratory animals, to fill this gap, the current study measured serum levels of GDH2 and SIRT4 in patients with focal epilepsy (FE) and generalized epilepsy (GE) and compared them to a control group. The study further investigated how alterations in these enzymes might contribute to epileptogenesis, affect seizure progression, and function as potential diagnostic biomarkers. In addition, electrolyte levels (Na, K, and Cl) and trace elements (Zn, Cu, and Se) were assessed to determine their potential effects on seizure frequency and epilepsy progression.

II. Materials and Methods

A. Study Population

The study was approved by the Human Research Ethics Committee under reference number SU2025HREC/30, with informed consent from all patients and their families. This study was conducted in the Neurology Department of Rizgare Teaching Hospital and the Epilepsy Clinic of Erbil Teaching Hospital in Erbil, Kurdistan Region of Iraq. Blood samples were collected between May and November 2024 from 60 epileptic male and female patients (30 with FE and 30 with GE) and a control group (30 samples). The ages of the groups ranged from 16 to 60 years. All patients in this study were diagnosed with epilepsy, either clinically or by electroencephalography. The study included patients with concurrent febrile epilepsy since childhood, those with a family history of epilepsy (more than one family member has epilepsy), and those who had suffered a head injury. All patients in both groups had uncontrolled seizures, and their medications and dosages were modified in accordance with the severity of the seizures. For the FE group (Carbamazepine) and for GE (Convulex and Levetiracetam), patients with other medical conditions were excluded from the study. The hospital's ethics committee approved the study protocol.

B. Blood Sample Collection

The blood taken from the veins of patients and the control group was approximately 5 mL of blood. The sample was centrifuged at 6000 rpm for 10 min to obtain the serum, and the sample was kept frozen at −40°C until analysis.

C. Measurements of GDH2, SIRT4

An enzyme-linked immunosorbent assay kit from Sunlong Biotech Co., LTD., was used to measure the levels of

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GDH2 and SIRT4 in the serum of the control and epilepsy groups. The assay applies the quantitative sandwich enzyme immunoassay method, and the optical density was measured spectrophotometrically at a wavelength of 450 nm. GDH2 and SIRT4 values in the examined samples are expressed in pg./mL.

D. Measurements of Electrolytes and Trace Elements

Electrolytes in blood serum were measured using the Sensa Core ST-200 Pro Electrolyte Analyzer, a fully automated microprocessor-controlled electrolyte system that used the ST-200 pro/plus/CL reagent pack for the determination of Na, K, and Cl. The trace elements (Zn, Cu, and Se) were measured by inductively coupled plasma-mass spectrometry.

E. Statistical Analysis

Statistical analyses were done using GraphPad Prism 9.0. Anderson-Darling, D'Agostino and Pearson, Shapiro–Wilk, and Kolmogorov–Smirnov tests were utilized to assess the normal distribution of variables. Ordinary one-way analysis of variance was applied to compare the data among groups, and the data were expressed as mean ± standard error of mean. Values of $p < 0.05$ were considered statistically significant. The curve receiver operating characteristic calculates the area under the curve (AUC) for diagnostic accuracy in epilepsy patients. However, for the application of artificial neural networks (ANNs), the neural network model was constructed, and its accuracy was tested using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 25's multilayer perceptron (MLP) module. A backpropagation learning algorithm that uses gradient descent to adjust weights to lower the error function was used to train MLP neural networks.

III. Results

This study was conducted on 90 men and women, who were divided into three groups: A control group with 30 individuals, and two groups of patients diagnosed with epilepsy (30 patients with,FE, and 30 patients with generalized epilepsy (GE). The levels of GDH2 and SIRT4 enzymes are summarized in Table I and Fig. 1. The mean value and standard error for GDH2 were 974.2 ± 13.06 for the FE group and 896.6 ± 21.23 for the GE group, both of which were significantly decreased compared to the control group's mean value of 1095 ± 29.85. In contrast to GDH2, the level of SIRT4 is elevated considerably in the FE group (2067 ± 53.91) and GE group (2326 ± 73.40) compared to the control group (1620 ± 54.50).

Table II and Fig. 2a-c illustrate levels of Na, K, and Cl, which show a decrease in sodium levels in the FE group (130.0 ± 2.823) and the GE group (123.4±3.367) as compared to the control group (157.8 ± 4.865). K and Cl exhibited no significant differences, with values of (4.251 ± 0.06353 and 4.399 ± 0.1287) for K and (100.7 ± 0.5352 and 100.1 ± 0.7671) for Cl in both the FE and GE, while in the control groups, they were (4.663 ± 0.1833 and 103.1 ± 2.040) for K and Cl, respectively.

TABLE I
Mean±Standard Error of the Mean of GDH2 and SIRT4 in Control and Patient Groups (Focal Epilepsy and General Epilepsy)

Parameters	GDH2 (pg/mL)	p-value	p*	SIRT4 (pg/mL)	p-value	p*
Control	1095±29.85			1620±54.50		
FE	974.2±13.06	0.0007		2067±53.91	<0.0001	
GE	896.6±21.23	<0.0001	0.0431	2326±73.40	<0.0001	0.0100

Where P: Difference between control and patient groups (FE, GE), *p**: Difference between FE and GE. FE: Focal epilepsy, GE: Generalized epilepsy

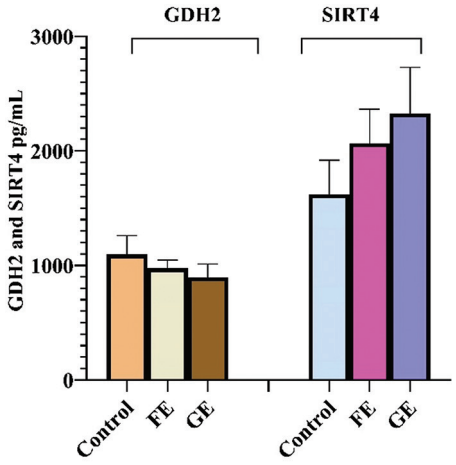

Fig. 1. Levels of GDH2 and SIRT4 in control and epileptic patient groups.

Table III and Fig. 2d and f indicate a significant decrease in both Zn and Se levels (57.57 ± 1.410, 6.020 ± 0.3093) for the FE group and (55.75 ± 2.098, 5.747 ± 0.1617) for the GE group when compared to the control group (105.7 ± 5.842 and 13.75 ± 0.1529) for zinc and selenium, respectively. Unlike Zn and Se, Cu levels in the FE group (86.64 ± 2.534) and GE group (77.20 ± 3.974), as shown in Table III and Fig. 2e, did not differ significantly from the control group (78.46 ± 2.654).

ROC analysis, as shown in Fig. 3a and b, was performed to evaluate the sensitivity and specificity of GDH2, which revealed an AUC of 0.8092, and SIRT4, which showed an AUC of 0.8956. Both markers are elevated above normal levels and may represent promising biomarkers for epilepsy. The AUC values for the electrolytes were 0.8673 for sodium (Na), 0.579 for potassium (K), and 0.601 for chloride (Cl) as illustrated in Fig. 3 (c, d and e). The AUC values for the trace elements (Zn, Se, and Cu) were 0.9711, 1, and 0.544, respectively as demonstrayed in Fig. 3 (f, g and h).

Table IV shows the cross-entropy error for both training and test samples. Table IV shows that the predication made using these samples was inaccurate (0.0%), and the cross-entropy error for both the training and test samples was 0.006. A decrease in the error function of the test samples was observed as the discrimination process continued until 27 consecutive steps were obtained. Fig. 4a shows the network diagram used by SPSS to predict the differential outcome (healthy = 0, epilepsy = 1) from four biochemical tests. The

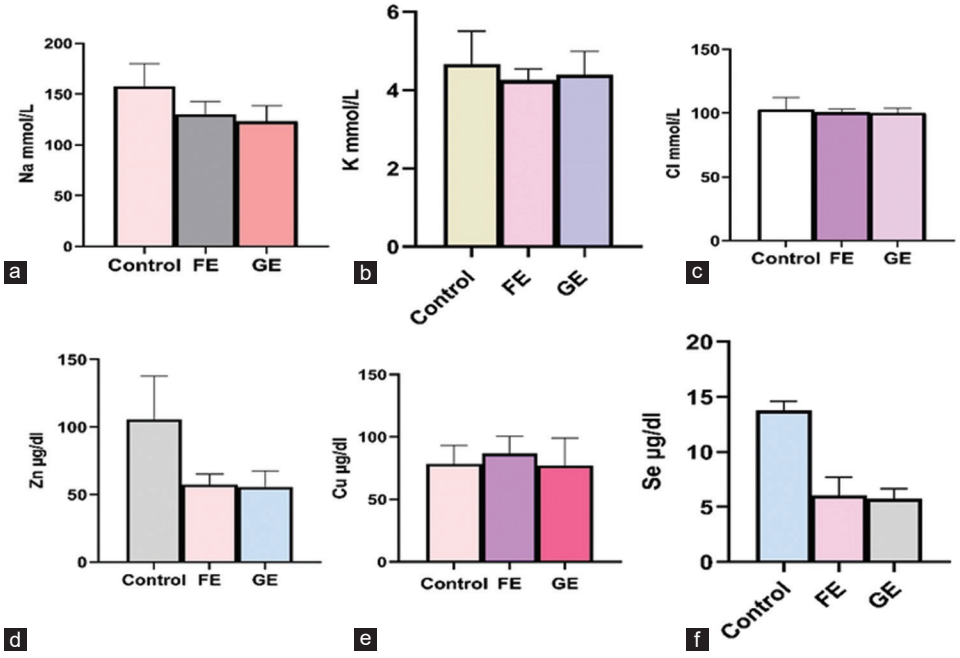

Fig. 2. Levels of (a) Sodium (b) Potassium, (c) Chloride, (d) Zinc, (e) Copper, (f) Selenium, in control and epileptic patient groups.

TABLE II
Mean±Standard Error of Electrolyte (Na, K, and Cl) in Control, Focal and General Epileptic Patient Groups

Parameters	Na	p-value	p*	K	p-value	p*	Cl	p-value	p*
Control	157.8±4.865			4.663±0.1833			103.1±2.040		
FE	130.0±2.823	0.003		4.251±0.06353	0.085		100.7±0.5352	0.4016	
GE	123.4±3.367	<0.0001	0.3236	4.399±0.1287	0.352	0.718	100.1±0.7671	0.2494	0.948

Where p: Difference between control and patient groups (FE, GE), *P**: Difference between FE and GE. FE: Focal epilepsy, GE: Generalized epilepsy

TABLE III
Mean±Standard Error of Zn, Cu, and SE in Control, Focal and General Epileptic Patient Groups

Parameters	Zn	p-value	p*	Cu	p-value	p*	Se	p-value	p*
Control	105.7±5.842			78.46±2.654			13.75±0.1529		
FE	57.57±1.410	<0.0001		86.64±2.534	0.1304		6.020±0.3093	<0.0001	
GE	55.75±2.098	<0.0001	0.934	77.20±3.974	0.7756	0.102	5.747±0.1617	<0.0001	0.655

Where p: Difference between control and patient groups (FE, GE), p*: Difference between FE and GE. FE: Focal epilepsy, GE: Generalized epilepsy

TABLE IV
Model Summary of Artificial Neural Networks

Model Summary		
Training	Cross-entropy error cross	0.006
	Percentage incorrect predictions	0.0%
	Stopping rule used	Training error ratio criterion (0.001) achieved
	Training time	0:00:00.00
Testing	Cross-entropy error	0.004
	Percentage incorrect predictions	0.0%

Dependent variable: Case

diagram shows two output nodes reflecting the epilepsy and health categories, four hidden layers, and eight input nodes.

IV. Discussion

This study of GDH2 enzyme levels in epilepsy patients showed a significant decrease in GDH2 levels in both epilepsy groups. GDH2 is a key enzyme that links metabolic processes (anabolism and catabolism) and is localized in mitochondria, primarily in astrocytes, where it catalyzes the oxidation of glutamate via the Krebs cycle to alpha-ketoglutarate. A decrease in GDH2 levels may indicate mitochondrial dysfunction, which can contribute to seizures, particularly recurrent or prolonged seizures.

The activity of the enzyme GDH2 depends on ADP for its function in supporting glutamate oxidation and ATP production in mitochondria. Therefore, impairing GDH2 activity causes a cascade of metabolic disturbances, including altered ATP/ADP ratios during neurological stress such as seizure and impaired glutamate oxidation and ATP production in astrocytes, which require high energy for synaptic glutamate clearance (Andersen, 2025). Under normal physiological conditions, low concentrations of glutamate are directed toward glutamine conversion, and higher concentrations toward the oxidation pathway in

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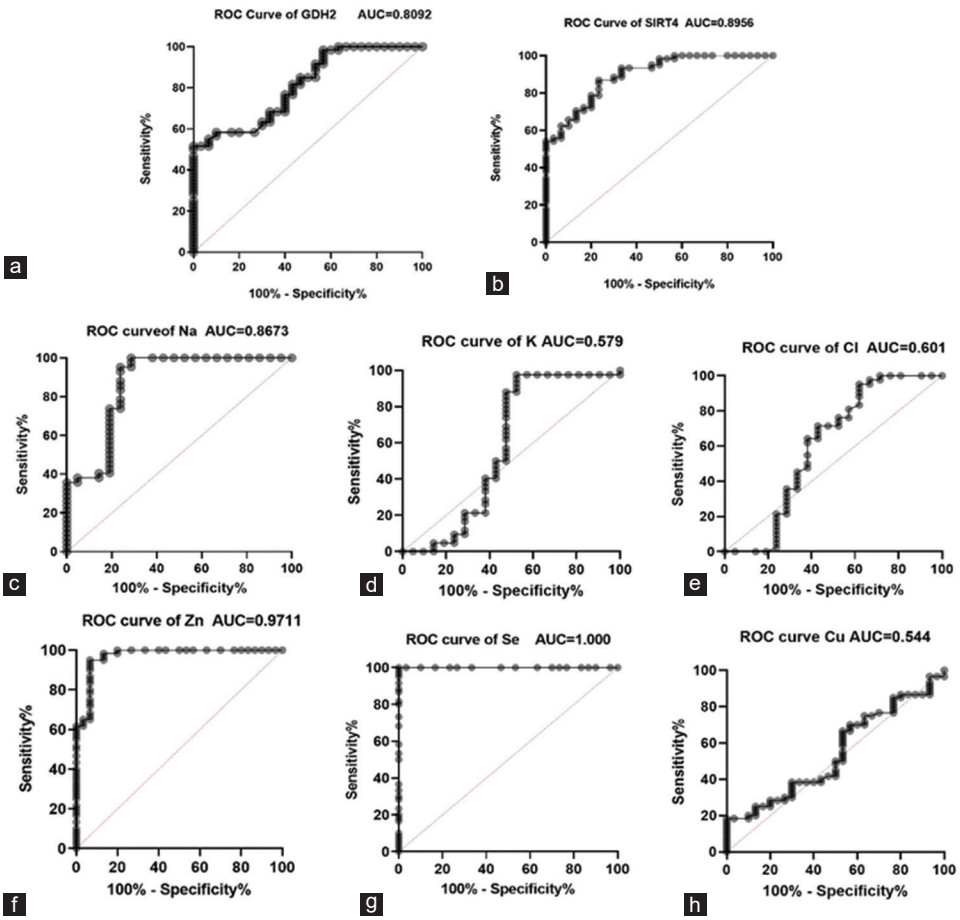

Fig. 3. Receiver operating characteristic of (a) GDH2, (b) SIRT4, (c) Na, (d) K, (e) Cl, (f) Zn, (g) Se, and (h) Cu.

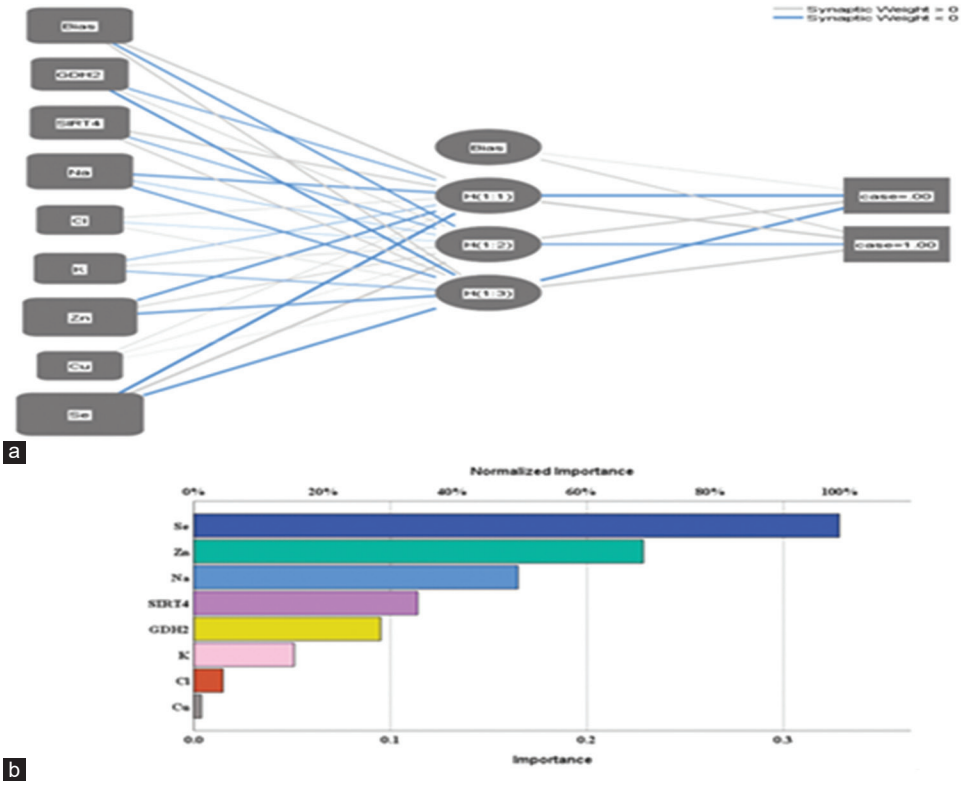

Fig. 4. (a) Network diagram, (b) Importance of independent variable chart.

mitochondria. When GDH2 enzyme activity is impaired, this process is significantly disrupted, resulting in altered activity significantly affects glutamate clearance (Andersen and Schousboe, 2023). Disruption of GDH2 activity significantly affects glutamate homeostasis, as this enzyme plays a key role in removing excess glutamate from synaptic clefts. This disruption may lead to excitotoxicity, manifested as excessive ion influx, particularly through the influx of Ca2+ and Na+ via overactive glutamate receptor channels. This ionic imbalance promotes mitochondrial calcium release and potential pathways (Yalçın and Colak, 2020). GDH2 is suggested to play a role in nuclear activities and is involved in the compartmentalized metabolism of glutamate in the brain, potentially aiding its metabolic and neuronal transmission functions. Its presence in astrocytes is hypothesized to facilitate the management of high glutamate levels during excitatory transmission. Dysregulation of GDH2 may lead to disturbance in energy metabolism and glutamate excitotoxicity (Plaitakis, et al., 2017).

The results of SIRT4 levels shown in Table I and Fig. 1 show elevated levels in both epilepsy groups compared to the control group, which had lower levels. These elevated SIRT4 levels are consistent with its neuroprotective effect in the brain, as they rise in response to excitotoxicity. This is consistent with what was indicated by (Betsinger and Cristea, 2019), where high levels of glutamate in brain synapses impede efficient neurotransmission and lead to excitotoxicity, causing cell death. Therefore, glutamate transport within the brain is crucial, as SIRT4 enhances glutamate uptake and mitigates neurotoxicity. SIRT4 thus protects neurons and regulates the growth of glial cells in the brain. This neuroprotective effect has important clinical value in the prevention and treatment of epilepsy.

The SIRT4 is predominantly located in mitochondria and functions in biological activities such as glutamine metabolism and fatty acid metabolism. SIRT4 regulates metabolism and energy status in many processes in the brain, such as preventing neuronal cell damage and information transmission between neurons, which are dependent on ATP. Thus, SIRT4 is thought to play an important role in brain function. Inhibiting glutamine metabolism in cell mitochondria, reducing mitochondrial oxidative capacity, and reducing ATP production (Zhu, Xiang and Zeng, 2021). SIRT4 expression is increased in response to excitotoxic challenges associated with epilepsy. Where neurons have high metabolic demands and are more susceptible to oxidative stress, SIRT4 levels are significantly elevated in brain tissue, and this upregulation appears to be a critical mechanism for responding to stress (Fagerli, et al., 2022).

Table II and Fig. 2a show that the level of the epileptic groups (FE and GE) exhibited a significant decrease in Na level compared to the control group. Electrolyte disturbances can lead to seizures and neurological complications. Abnormal levels of electrolytes in the blood, resulting from diseases such as kidney failure or dehydration, may interfere with neural electrical activity, affecting brain metabolism and increasing the risk of seizures. Seizures can result from excessive, erratic, and uncontrollable electrical activity caused by intracellular ions such as sodium. A common cause of seizure activity is hyponatremia, and the incidence of seizures rises dramatically when serum sodium levels fall (Aizaz, Khan, and Tiwar, 2024). Because ionotropic glutamate receptors show increased permeability to sodium ions and increased activity, which raises extracellular glutamate concentrations in the brain and causes epileptic seizures, changes in sodium currents may result in aberrant neural activity (Sar, et al., 2023).

Table II and Fig. 2 show the potassium and chloride values for both groups. The data did not reveal any significant differences in potassium and chloride levels between the patient and control groups. This indicates that the levels remained within the normal range. Several factors influence serum electrolyte levels, including kidney function, acid-base balance, hydration status, and the effects of antiepileptic drugs.

Some trace elements, such as zinc and selenium, possess antioxidant properties and, when their concentrations are maintained at stable levels, inhibit the onset of epilepsy by participating in and modulating specific signaling pathways. Furthermore, copper and other trace metals can enhance the generation of oxygen-free radicals, expedite neurotoxicity, and increase neuronal necrosis, potentially increasing vulnerability to epilepsy (Liu, et al., 2023). The results in Table III and Fig. 2a show that Zn levels are lower in epileptic patients than in the control group. Zinc influences the enzyme (GAD) glutamic acid carboxylase, which is required for the synthesis of GABA, an inhibitory neurotransmitter whose shortage can cause epileptic conditions. Zinc reduces the activation of type A GABA receptors. Changes in Zn levels increase the frequency of seizures, and limiting the amount present in living organisms decreases the activity of enzymes that rely on them, resulting in cell death (Aman-Mohammady, et al., 2023). Zn is a main component of SOD, an antioxidant enzyme that detoxifies oxidative radicals in the intracellular environment, whose accumulation causes neuronal membrane instability and epileptogenesis. This effect of SOD requires zinc. In addition, the neuromodulatory actions of zinc are hypothesized to be proconvulsant or anticonvulsant (Ran, et al., 2024). Antiepileptic drugs, such as carbamazepine, levetiracetam, and others, may contribute to variations in serum zinc levels. This difference is due to increased absorption of minerals from the gastrointestinal tract or decreased excretion from the body. And its effect on antioxidants, oxidative stress status, and the type of treatment the patients are receiving, whether it is monotherapy or combination therapy. However, the role of zinc in seizures is still controversial (Eissa, et al., 2020).

Table III and Fig. 2a show that the copper levels in the three groups were within the normal level of the element, and there were no significant differences. Both copper deficiency and excess can have major consequences for brain function, leading to neurological disorders. Copper ions can cause epileptic seizures by blocking Mg^{2+}-ATPase and Na^{+}-K^{+}-ATPase, altering the Na^{+}-K^{+} balance, and resulting in further seizures. Furthermore, copper's redox activity can generate hazardous reactive oxygen species (ROS), which

can injure the brain (Chen, et al., 2023). While pointing to a decrease in Se level in the epileptic patient group compared to the control group, as illustrated in Table III and Fig. 2b. Decreased blood serum selenium levels in epilepsy patients are primarily caused by increased consumption of selenium as an antioxidant to neutralize the oxidative stress and neuronal damage associated with seizures, rather than the epilepsy itself causing a direct depletion of the mineral. This overuse of selenium as part of the body's antioxidant defense system to combat ROS can lead to lower circulating levels. Both selenium deficiency and excess can be detrimental, highlighting the importance of maintaining a balanced intake. Selenium protects cells from oxidative stress by neutralizing ROS and free radicals. It is a key component of selenoproteins (Dlugosz, et al., 2025).Fig. 3 shows the ROC analysis of both GDH2 and SIRT4 enzymes, where the AUC showed values above normal levels. This indicates their active role in epilepsy and perhaps the possibility of using these two enzymes as effective biomarkers in the diagnosis of epilepsy. Also showed the AUC values for electrolytes and trace elements and indicated that sodium, zinc, and selenium all have high values and may serve as diagnostic biomarkers for epilepsy.

The model contents, displayed in Table IV, support the conclusions of the exclusion sample and the practice (and test) samples. The cross-entropy error is provided for each of the training and test samples, and it is the error function removed by the network during the training stage. The low value (0.006) of this inaccuracy represents the model's ability to predict the outcome of the trajectory. The cross-entropy variance is smaller for the exclusion sample in comparison to the training and test datasets, demonstrating that the network model learned to generalize from the trend and was not biased by the training data. The finding demonstrates the role of the test sample in preventing overtraining (Nair et al., 2021; AbuAlrob, Itbaisha, and Mesraoua, 2025). Fig. 4b indicates the influence of every measured parameter in the model of ANN in terms of proportional and normalized impact, and also depicts the importance of the parameters; it means how sensitive the model is to the alteration in each input parameter. From the result in the figure, it is apparent that variables related to distinguishing patients from others, the Se, Zn, Na, SIRT4, and GDH2, have the highest effect on how the network classifies patients in terms of epilepsy and health, while the K, Cl, and Cu have less effect. This relationship between epilepsy, some enzyme activity, and some elements may reflect a combination of oxidative stress, energy metabolism disorder, and long-term neurotoxicity, especially under chronic seizure conditions; enzyme profiling may therefore be a helpful biomarker approach in diagnosing and monitoring epilepsy (Wang et al., 2021; Milne-Ives, et al., 2025).

V. Conclusion

The study confirmed the role of some enzymes, electrolytes, and trace elements in the pathophysiology of epilepsy and its diagnosis. Compared with controls, the results demonstrated a pronounced reduction in serum GDH2 and a marked elevation in SIRT4 across all patient groups. In addition, the levels of Na in the patients were significantly less than in the control group, while there were no differences in levels of K and Cl. Furthermore, there was a decrease in the level of trace elements Zn and Se, in contrast to Cu, which showed no change in the level. Based on these results, the levels of enzymes and elements may be able to be used as parameters for detecting the state of patients or as biomarkers, and more research is needed. From the result in ANNs, it is apparent that the variables related to distinguishing patients from healthy ones, the Se, Zn, Na, SIRT4, and GDH2, have the most effect on how the network classifies patients in terms of epilepsy and health, while the K, Cl, and Cu have less effect.

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