# Autoantibodies in epileptic patients indicate the autoimmune epilepsy

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**Background**
Epilepsy is a multifaceted neurological condition marked by recurring seizures. Delving into the functions of VGKC (voltage-gated potassium channels), AMPA GluR3 (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit 3), GAD65 (glutamate decarboxylase 65), and LGI1 (leucine-rich glioma-inactivated 1) in epilepsy yields significant perspectives into the fundamental immunological mechanisms of this disorder.
**Objective**
The study target was to assess the clinical significance of neural autoantibody biomarkers in patients with epilepsy and determine the types of autoimmune epilepsy.
**Materials and methods**
A case–control study that comprised 50 epilepsy patients (33 males and 17 females) and 40 controls (21 males and 19 females), the patients and controls attending Basrah Teaching Hospital between November 2022 and March 2023, Basrah City, Southern Iraq. The control age ranged from 2 to 62 years and patients' age 2–68 years were considered the control group in this study. The VGKC, AMPA GluR3, GAD65, and LGI1 were measured using the sandwich ELISA technique.
**Results and conclusion**
There is no statistically significant difference between the patients and control group for VGKC, GAD65, and LGI1 ($P$=0.460, $P$=0.061, and $P$=0.440, respectively), while there is a statistically significant difference between the patients and the control group for AMPA GluR3 ($P$=0.012). GAD65 appeared an elevation in epilepsy patients but was not statistically significant. This upregulation may contribute to the hyperexcitability observed in the epileptic brain and potentially play a role in seizure generation and propagation.

**Keywords:**
AMPA GluR3 (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit 3), epilepsy, GAD65 (glutamate decarboxylase 65), LGI1 (leucine-rich glioma-inactivated 1), VGKC (voltage-gated potassium channels)

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## Introduction

Epilepsy is caused by hyperexcitability and an imbalance between excitation and inhibition, which results in seizures. According to the World Health Organization (WHO), this disease is a neurological condition that affects about 50 million people worldwide [1]. It was discovered that 6.9 per 1000 people in Arab nations have epilepsy at some point in their lifetime. The incidence is 89.5 per 100 000 on average. Parental consanguinity, a family history of epilepsy, and a history of prenatal illnesses or injuries were the most commonly found risk factors [2].

Based on the etiology, epilepsy is divided into genetic, structural, metabolic, infectious, unknown, or immune. In recent years, the idea of 'autoimmune epilepsy (AE)' has gained more acceptance as an etiology and many patients who present with encephalopathy and/or seizures also have anti-neuronal antibodies present [3]. As a result, AE is now designated as a separate organization in the International League Against Epilepsy (ILAE) most recent classification [4]. The existence of various types of linked neuronal autoantibodies plays a major role in the definition of AE, and the current understanding of the related clinical manifestations has evolved [5]. While the exact cause of autoimmunity is frequently elusive, certain patients might harbor an underlying malignancy capable of inciting a paraneoplastic immune reaction [6]. Also, the concept of immune-mediated epileptogenesis may drive new therapy trials that concentrate on the main reason for seizure

development and provide patients with new treatment options [7].

Whenever prodromal signs appear, prodromal symptoms can include a fever, headache, dizziness, sleeplessness, or an upper respiratory infection. Overlapping symptoms of memory problems, psychological issues, or changed mental status are all associated with autoimmune epilepsy [8]. There are some neural autoantibodies such as AMPA GluR3, VGKA, GAD65, and LGI1 associated with autoimmune epilepsy but the direct pathogenesis of these antibodies is not clear yet. VGKC, a group of potassium channels, modulates the resting membrane potential and shapes the action potential duration in neurons. Dysfunction of autoantibodies targeting VGKC has been associated with limbic encephalitis and seizures in certain epilepsy cases [9]. AMPA GluR3 is a subunit of AMPA receptors, which mediate fast excitatory neurotransmission in the brain. Alterations in AMPA receptor expression and function have been observed in various epilepsy types, suggesting their involvement in epileptogenesis [10]. GAD65 is an enzyme responsible for synthesizing the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Autoantibodies against GAD65 have been found in certain epilepsy patients, particularly those with temporal lobe epilepsy [11]. LGI1 is a secreted protein that interacts with synaptic proteins, which play a role in synapse development and function [12]. Mutations in the LGI1 gene have been linked to autosomal-dominant lateral temporal lobe epilepsy, suggesting a critical role for LGI1 in the regulation of neuronal excitability and synchronization [13].

The goal of this study is to identify autoimmune epilepsy in different forms of epilepsy and evaluate the clinical significance of neural autoantibodies as biomarkers in epilepsy patients.

## Materials and methods

### The study design

A case–control study was conducted from November, 2022 to March, 2023 on 90 participants (51 males, 39 females), 50 epilepsy patients (33 males,17 females), and 40 (21 males,19 females) control. The patients included 24 drug-resistant and 26 responders; their ages were between 2 and 60 years old, who attended Basra Teaching Hospital. Control ages range is from 2 to 62 years which were considered as control groups in this study in Basrah, Southern Iraq. We excluded patients with other autoimmune diseases, smoking patients, patients who take immune drugs, and stroke were excluded from participating in this study.

### Blood collection and test

Five milliliters of blood was collected by vein puncture in sterile gel tubes from all participant groups. Blood in a gel tube was left to clot at room temperature, then centrifuged at 3000 RPM for 5 min. VGKC and GAD65 were manufactured by SunLong company in China. LGI1 and AMPA GluR3 were manufactured by Biotechnology Company in China. Blood samples for the measurement of VGKC, AMPA GluR3, GAD65, and LGI1 were measured by the sandwich enzyme-linked immunosorbent assay (ELISA) technique. On the included Micro Elisa strip plate, standards or samples were added, and the particular antibodies were combined in the relevant Micro ELISA strip plate wells. Specific antibodies that had been HRP-conjugated were then added to each Micro ELISA strip plate well and incubated. While being washed, free pieces were eliminated. Tetra methyl benzidine (TMB) substrate solution was injected into each well. Only the wells containing horseradish peroxidase (HRP) conjugated initially turn blue after the stop solution has been added. At a wavelength of 450 nm, the optical density (OD) was calculated spectrophotometrically. It can be seen that the OD value was inversely related to the concentration by contrasting the OD of the samples to the standard curve.

### Ethical considerations

A written illustrative consent form was signed by all parents/caregivers of the participating patients. This study was performed according to the ethical rules for

**Table 1 Demographic characteristics of the study population**

| Variable | Category | | *P* value |
|---|---|---|---|
| | Patient | Control | |
| Sex | | | |
| Male | 33 (66.0%) | 21 (52.5%) | 0.194* |
| Female | 17 (34.0%) | 19 (47.5%) | |
| Age group (year) | | | |
| ≤15 | 27 (54.0%) | 21 (52.5%) | 0.931** |
| 16-25 | 9 (18.0%) | 8 (20.0%) | |
| 26-35 | 7 (14.0%) | 6 (15.0%) | |
| 36–45 | 2 (4.0%) | 2 (5.0%) | |
| 46–55 | 2 (4.0%) | 0 (0.0%) | |
| ≥56 | 3 (6.0%) | 3 (7.5%) | |
| Total | 50 (100.0%) | 40 (100.0%) | |

*Chi-square test. **Fisher's exact test.

**Table 2 Differences in VGKC, AMPA GluR3, GAD65, and LGI1 levels according to category**

| Category | VGKC | AMPA GluR3 | GAD65 | LGI1 |
|---|---|---|---|---|
| Patient median (Min–max) | 1.4 (0.11–34.72) | 5.92 (0.62–16.42) | 61.53 (7.92–270.11) | 4.85 (1.32–11.93) |
| Control median (min–max) | 1.52 (0.39–8.33) | 1.51 (0.17–16.09) | 24.55 (9.09–209.04) | 4.29 (1.97–11.65) |
| *P* value* | 0.460 | 0.012 | 0.061 | 0.440 |

*Mann–Whitney U Test.

**Table 3 Differences in VGKC, AMPA GluR3, GAD65, and LGI1 levels according to the presence of drug resistance**

| Drug resistance | VGKC | AMPA GluR3 | GAD65 | LGI1 |
|---|---|---|---|---|
| Present median (min–max.) | 1.11 (0.11–31.05) | 7.30 (1.17–16.10) | 62.54 (8.63–270.11) | 4.35 (1.74–10.15) |
| Absent median (min–max.) | 1.55 (0.16–3472) | 5.09 (0.62–16.42) | 61.53 (7.92–250.81) | 5.69 (1.32–11.93) |
| *P* value* | 0.341 | 0.062 | 0.801 | 0.593 |

*Mann–Whitney U Test.

medical research involving human participants of the Declaration of Helsinki (1964). Ethical approval was received from the ethics and research committee of the Department of Medical Laboratory Technology, College of Health and Medical Technology, Southern Technical University, Basrah, Iraq.

### Statistical analysis

Statistically significant differences were determined using SPSS (version 26). All statistical analyses were performed using a statistical package for Data were expressed median<AQ: Pls check the sentence for clarity>. *P* values less than or equal to 0.05 ($P \leq 0.05$) is considered significant. We used nonparametric tests such as the Chi-square test, Fisher's exact test, Mann-Whitney U test, and Kruskal–Wallis test in our research.

## Results

Table 1 recorded the most cases of epilepsy recorded among male groups: 33 (66%) versus 17 (34%) for female groups from total study patients; 50 (100%); 21 (52.5%) for male group versus 19 (47.5%) for the female group from total study controls. Statistically, this difference was nonsignificant ($P=0.194$). The highest age group of patients with epilepsy was ≤15 years, with 27 (54%) of the total study patients being 50 (100.0%), while fewer cases of epilepsy patients appeared at 36–45 and 46–55 years, with 2 (4%) for each from total study cases (50, 100.0%). Statistically, this difference was nonsignificant ($P=0.931$).

Table 2 shows that there was no statistically significant difference between the patient and control groups for VGKC, GAD65, and LGI1 ($P=0.460$, $P=0.061$, and $P=0.440$, respectively), while there was a statistically significant difference between the patient and control groups for AMPA GluR3 ($P=0.012$).

In Table 3, there is no statistically significant difference between the drug resistance and nondrug resistance for VGKC, AMPA GluR3, GAD65, and LGI.

Table 4 shows that there is no statistically significant difference in VGKC, AMPA GluR3, GAD65, and LGI1 levels among the age groups of patients.

**Table 4 Association between age groups of epilepsy patients and immunological biomarkers**

| Age group (Year) | VGKC | AMPA GluR3 | GAD65 | LGI1 |
|---|---|---|---|---|
| ≤15 | | | | |
| N | 27 | 27 | 27 | 27 |
| Median | 1.60200 | 7.18500 | 82.13600 | 3.93000 |
| Minimum | 0.193 | 0.626 | 15.626 | 1.322 |
| Maximum | 34.725 | 16.422 | 270.110 | 11.398 |
| 16-25 | | | | |
| N | 9 | 9 | 9 | 9 |
| Median | 0.57100 | 5.95600 | 30.41400 | 6.38100 |
| Minimum | 0.113 | 1.121 | 8.639 | 1.869 |
| Maximum | 19.999 | 16.104 | 139.230 | 10.157 |
| 26-35 | | | | |
| N | 7 | 7 | 7 | 7 |
| Median | 1.10200 | 7.82900 | 38.21900 | 3.60100 |
| Minimum | 0.189 | 0.998 | 7.924 | 2.963 |
| Maximum | 13.563 | 10.238 | 138.870 | 11.937 |
| 36–45 | | | | |
| N | 2 | 2 | 2 | 2 |
| Median | 0.83450 | 1.08850 | 97.13950 | 5.18700 |
| Minimum | 0.167 | 0.817 | 29.189 | 4.066 |
| Maximum | 1.502 | 1.360 | 165.090 | 6.308 |
| 46–55 | | | | |
| N | 2 | 2 | 2 | 2 |
| Median | 0.43250 | 3.94400 | 95.90050 | 8.19950 |
| Minimum | 0.193 | 3.146 | 11.221 | 6.782 |
| Maximum | .672 | 4.742 | 180.580 | 9.617 |
| ≥56 | | | | |
| N | 3 | 3 | 3 | 3 |
| Median | 1.73300 | 1.21400 | 87.90300 | 5.85200 |
| Minimum | 1.401 | 1.179 | 13.402 | 4.446 |
| Maximum | 2.501 | 1.811 | 143.120 | 6.292 |
| *P*-value | 0.198 | 0.073 | 0.882 | 0.383 |

*Kruskal–Wallis test.

**Table 5 Differences in VGKC, AMPA GluR3, GAD65, and LGI1 levels according to sex**

| Sex | VGKC | AMPA GluR3 | GAD65 | LGI1 |
|---|---|---|---|---|
| Male median (min–max) | 1.31 (0.11–31.05) | 5.44 (0.99–16.10) | 30.41 (7.92–270.11) | 5.07 (1.74–11.9) |
| Female median (min–max.) | 1.60 (0.16–34.72) | 9.22 (0.62–16.42) | 82.13 (13.40–250.81) | 4.09 (1.32–11.39) |
| *P*-value* | 0.219 | 0.301 | 0.231 | 0.667 |

*Mann–Whitney U Test.

**Table 6 Differences in VGKC, AMPA GluR3, GAD65, and LGI1 levels according to type of epilepsy**

| Type of Epilepsy | VGKC | AMPA GluR3 | GAD65 | LGI1 |
|---|---|---|---|---|
| Focal median (min–max.) | 1.39 (0.11–32.15) | 5.76 (0.62–16.10) | 43.46 (7.92–270.11) | 4.62 (1.32–11.93) |
| Generalized median (min–max.) | 2.98 (0.30–34.72) | 9.22 (1.17–16.42) | 87.90 (16.02–250.81) | 5.59 (2.68–11.39) |
| *P* value* | 0.142 | 0.257 | 0.378 | 0.685 |

*Mann–Whitney U Test.

Table 5 shows that there is no statistically significant difference in VGKC AMPA GluR3, GAD65, and LGI1 levels between males and females.

Table 6 shows that there is no statistically significant difference in VGKC, AMPA GluR3, GAD65, and LGI1 levels between focal and generalized epilepsy.

## Discussion

The study by Fisher RS *et al.* (2014) demonstrated that the most cases of epilepsy were recorded among the male group 33 (66%) versus 17 (34%)for the female group from the total study patients (50, 100%), and (male 21(52.5%) versus female 19 (47.5%)) from the control group. Statistically, this difference was nonsignificant ($P$=0.194) [14]. Our results agree with a previous study of Savic *et al.*, 2014, which shows the prevalence of epilepsy is slightly higher in males compared with females, with rates of 50.7% for males and 46.2% for females. This difference can be attributed to the higher occurrence of partial epilepsies in males [15]. In the current study, the highest age group of patients with epilepsy was ≤15 years (27, 54%) from the total study patients (50, 100.0%), while fewer cases of epilepsy patients appeared at 36–45 and 46–55 were 2(4%) for each one from total study cases 50 (100.0%)<AQ: Pls check for clarity of meaning>. Statistically, this difference was nonsignificant ($P$=0.931). Our results disagree with the study of Beghi and Giussani, 2018, which illustrated that the prevalence tends to increase with age, with peaks in the oldest age groups and socially deprived individuals [16]. Cabezudo-García *et al.* (2021) showed that epilepsy has a bimodal distribution according to age with peaks in the youngest individuals and the elderly. The increased incidence of seizures and epilepsy in the elderly can be attributed to the increase of age-related and aging-related epileptogenic [17]. Fiest *et al* .(2017) showed that the prevalence of epilepsy did not differ by age group, sex, or study quality. The active annual period prevalence, lifetime prevalence, and incidence rate of epilepsy were higher in low–to-middle-income countries. Epilepsies of unknown etiology and those with generalized seizures had the highest prevalence and this supported our results that epilepsy tends to be slightly more prevalent in males than in females [18]. The findings of our study indicated that partial seizures were the predominant seizure type (51.9%) in patients over 50 years old, followed by generalized seizures (37.5%), and unclassified seizures (10.6%). Sen *et al.* (2019) found that the results showed that partial seizures are the most common type of seizure (51.9%) in patients, followed by generalized (37.5%) and unclassified (10.6%). These results indicated that in the elderly Chinese population experience epilepsy is a relatively more dangerous period<AQ: Pls check the sentence for clarity of meaning>, and partial seizures were the most common seizure type [19]. Mohamed *et al.*, 2023, investigated the presence of NMDA-R, AMPA1-R, AMPA2-R, CASPR2, LGI1, GABAB-R, and GAD65 autoantibodies in serum samples of Egyptian patients with new-onset drug-resistant epilepsy. In this study, out of 25 patients with recently developing drug-resistant epilepsy, 4% patients had anti-N-methyl-D-aspartate (anti-NMDA) antibodies positive, and another 4% had anti-GAD 65 positive in both serum. Even though the remaining 23 patients tested negative for the other autoantibodies, 92% of them experienced seizure independence or a more than 50% reduction in seizure frequency after undergoing immunotherapy treatment, and 84% showed a noticeable improvement in seizure-associated symptoms [20].

## Conclusion

This study reported that AMPA GluR3 biomarker is statistically significant between the epilepsy patient group and the control, while there is no statistical

significance in GAD65, LGI1, and VGKC between the epilepsy patient group and the control. Furthermore, there is no statistically significant difference between these biomarkers and age group, drug resistance, sex, and type of epilepsy. The perspective studies will need to be confirmed in a later large-scale analysis (with a large sample size) to generalize the findings to the entire country.

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**Author Contribution:** NHA: Concept study design data, literature overview discussion; MKD: literature overview discussion, data analysis; HNN: Experiments working, collection statistical analysis. All authors contribute to the writing, editing, and approval of the final manuscript version.

**Declarations: Ethics approval and consent to participate:** Ethical approval was received from the ethics and research committee of the Department of Medical Laboratory Technology, College of Health and Medical Technology, Southern Technical University, Basrah, Iraq. Informed consent was obtained from all caregivers of participants.

**Consent for publication** Informed written consent was obtained from all the study participants.

**Availability of data and material:** Datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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#### Conflicts of interest

The authors declare there are no conflicts of interest.

## References

1 Lilleker JB, Biswas V, Mohanraj R. Glutamic acid decarboxylase (GAD) antibodies in epilepsy: diagnostic yield and therapeutic implications. Seizure 2014; 23:598–602.

2 Idris A, Alabdaljabar MS, Almiro A, Alsuraimi A, Dawalibi A, Abduljawad S, *et al.* Prevalence, incidence, and risk factors of epilepsy in arab countries: A systematic review. Seizure 2021; 92:40–50.

3 Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, *et al.* ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017; 58: 512–521.

4 Lapalme-Remis S, Nguyen DK. Neuroimaging of Epilepsy. Contin Lifelong Learn Neurol 2022; 28:306–338.

5 Yavuz ENV, Altındağ E, Tüzün E, Baykan B. Do the neurologists recognize autoimmune epilepsy well enough? What is the effect of the pandemic on this matter? Neurol Sci 2022; 43:5029–5037.

6 Aguilar-Castillo MJ, Cabezudo-García P, Ciano-Petersen NL, García-Martin G, Marín-Gracia M, Estivill-Torrús G, *et al.* Immune mechanism of epileptogenesis and related therapeutic strategies. Biomedicines 2022; 10:716.

7 Husari KS, Dubey D. Autoimmune epilepsy. Neurotherapeutics 2019; 16:685–702.

8 Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, *et al.* A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol 2016; 15:391–404.

9 Kalachikov S, Evgrafov O, Ross B, Winawer M, Barker-Cummings C, Boneschi FM, *et al.* Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. Nat Genet 2002; 30:335–341.

10 Lai Q, Li Q, Li X, Wang H, Zhang W, Song X, *et al.* GluR3B antibody was a biomarker for drug-resistant epilepsy in patients with focal to bilateral tonic-clonic seizures. Front Immunol 2022; 13:838389.

11 Christian CA, Reddy DS, Maguire J, Forcelli PA. Sex differences in the epilepsies and associated comorbidities: implications for use and development of pharmacotherapies. Pharmacol Rev 2020; 72:767–800.

12 Kegel L, Aunin E, Meijer D, Bermingham JR. LGI proteins in the nervous system. ASN Neuro 2013; 5:167–181.

13 Shin Y-W., Lee S-T., Shin J-W., Moon J, Lim J-A., Byun J-I., *et al.* VGKC-complex/LGI1-antibody encephalitis: clinical manifestations and response to immunotherapy. J Neuroimmunol 2013; 265:75–81.

14 Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, *et al.* ILAE official report: a practical clinical definition of epilepsy. Epilepsia 2014; 55:475–482.

15 Savic I. Sex differences in human epilepsy. Exp Neurol 2014; 259: 38–43.

16 Beghi E, Giussani G. Aging and the epidemiology of epilepsy. Neuroepidemiology 2018; 51:216–223.

17 Cabezudo-García P, Mena-Vázquez N, Ciano-Petersen NL, García-Martín G, Estivill-Torrús G, Serrano-Castro PJ. Prevalence of neural autoantibodies in epilepsy of unknown etiology: systematic review and meta-analysis. Brain Sci 2021; 11:392.

18 Fiest KM, Sauro KM, Wiebe S, Patten SB, Kwon C-S., Dykeman J *et al.* Prevalence and incidence of epilepsy: a systematic review and meta-analysis of international studies. Neurology 2017; 88:296–303.

19 Sen A, Jette N, Husain M, Sander JW. Epilepsy in older people. Lancet 2020; 395:735–748.

20 Mohamed H, Hemeda M, Gaber A, Zakieldine H, Bakr SI, Shehab A, *et al.* Neuronal autoantibodies in a sample of Egyptian patients with drug-resistant epilepsy. Egypt J Neurol Psychiatry Neurosurg 2023; 59:83.