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Short Communication

Neuroprotective and inflammatory biomarkers in pediatric drug-resistant epilepsy: Interplay between GDNF, IL-1β and vitamin D 25-OH

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Abstract

Drug-resistant epilepsy in pediatric patients is associated with neuroinflammation and neurodegeneration. Vitamin D 25-OH exerts neuroprotective effects, while glial cell line-derived neurotrophic factor (GDNF) and the proinflammatory cytokine interleukin-1β (IL-1β) are implicated in the mechanisms of neuroinflammation and epileptogenesis. The aim of this study was to investigate the relationship between vitamin D 25-OH, IL-1β, and GDNF levels with seizure severity and frequency in children with drug-resistant epilepsy. A cross-sectional study was conducted at Adam Malik Hospital, Medan, Indonesia, among children with drug-resistant epilepsy. Vitamin D 25-OH, IL-1β and GDNF levels were measured using enzyme-linked immunosorbent assay (ELISA). Epilepsy severity was assessed using the Hague Seizure Severity Scale (HASS), while seizure frequency was assessed using the Global Assessment of Severity of Epilepsy (GASE). The present study identified a significant correlation between GDNF levels and epilepsy severity, as measured by the HASS score (r=0.318; p=0.006). However, no significant correlation was observed between vitamin D 25-OH or IL-1β levels and epilepsy severity or seizure frequency (p>0.05). IL-1β levels correlated significantly with GDNF levels (r=0.525; p=0.001), but IL-1β did not directly correlate with seizure frequency or epilepsy severity. In conclusion, GDNF levels significantly correlated with epilepsy severity, suggesting that GDNF may serve as a potential biomarker for assessing epilepsy severity. However, further studies investigating the role of GDNF as a potential neurotrophic factor in the pathophysiology of epilepsy and its possible application as a therapeutic target are important.

Keywords: Drug-resistant epilepsy, biomarker, vitamin D 25-OH, IL-1β, GDNF

Introduction

Drug-resistant epilepsy is a complex neurological condition that significantly challenges treatment, particularly in children [1,2]. Approximately 20–30% of pediatric epilepsy cases do not respond to therapy with two or more anticonvulsant medications [3-5]. Characterized by uncontrollable seizures, this condition greatly impacts the quality of life for patients and their

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families [6]. Previous studies have indicated that the high frequency and severity of seizures contributed to a markedly reduced quality of life, highlighting the urgent need for more effective therapeutic strategies [7-9].

Neuroinflammation plays a crucial role in the pathogenesis of epilepsy by activating microglia and astrocytes, which subsequently release inflammatory mediators that lead to neuronal hyperactivation [10-12]. Proinflammatory cytokines, such as interleukin 1β (IL-1β), are associated with increased severity of epilepsy [13,14]. In contrast, neurotrophic factors, such as glial cell-line-derived neurotrophic factor (GDNF), exhibit neuroprotective effects that could decrease seizure frequency and enhance cognitive function [15,16].

Vitamin D 25-OH exhibits neuroprotective and anti-inflammatory effects and has been associated with seizure frequency in epilepsy patients, with deficiency associated with worse disease progression [17,18]; therefore, it is essential for maintaining central nervous system function [17,19,20]. Vitamin D deficiency is prevalent among epilepsy patients undergoing long-term anticonvulsant therapy, potentially exacerbating neuroinflammation and affecting neuronal activity [21,22]. Moreover, abnormal levels of biomarkers such as IL-1β and GDNF are known to influence the severity of drug-resistant epilepsy [14,23,24]. Therefore, in the present study, further analysis of the role of vitamin D 25-OH levels and its impact on inflammatory biomarkers, such as IL-1β and GDNF, is crucial for understanding its relationship with epilepsy severity.

The aim of this study was to investigate the relationship between vitamin D 25-OH, IL-1β, and GDNF levels and seizure severity and frequency in children with drug-resistant epilepsy. The findings are expected to inform the development of more effective therapies for drug-resistant epilepsy and enhance patient quality of life through improved prevention and treatment strategies. Furthermore, the results may support the incorporation of vitamin D 25-OH supplementation, anti-inflammatory agents, or neuroprotective therapies into treatment regimens for the drug-resistant epilepsy population by providing insights into treatment responses based on seizure frequency and severity.

Methods

Study design and setting

A cross-sectional study was conducted at Adam Malik Hospital, Medan, Indonesia, to analyze the relationship between vitamin D 25-OH, IL-1β, and GDNF levels and seizure frequency and epilepsy severity in children with drug-resistant epilepsy. Eligible patients based on inclusion and exclusion criteria were assessed to determine the seizure frequency and epilepsy severity. Laboratory examinations were conducted to measure vitamin D 25-OH, IL-1β, and GDNF levels.

Sampling strategy and criteria

The target population for the present study comprised children with drug-resistant epilepsy attending the Pediatric Neurology outpatient clinic at Adam Malik Hospital, Medan, Indonesia, between January and April 2024. A purposive sampling method was employed, with inclusion criteria: (1) children aged 1 to 18 years; (2) those who had been on anti-seizure medications for over 24 weeks but continued to experience seizures in the last 12 weeks; and (3) those who had not taken vitamin D 25-OH supplements in the previous 24 weeks. Exclusion criteria included children with heart, kidney, or liver diseases, severe nutritional deficiencies, or other non-neurologic chronic conditions. A total of 73 patients met these criteria within the study period and were included in the study.

Data collection

Demographic data (age and sex) and clinical data (seizure frequency and epilepsy severity) were assessed from all patients with drug-resistant epilepsy after receiving consent from the parents. The patients were categorized into children (0–9 years old) and adolescents (10–18 years old) to facilitate age-group-specific analysis. Seizure frequency was assessed by estimating the frequency over one month and by using components of the Global Assessment of Severity of Epilepsy (GASE) questionnaire, completed by the patient's parents. Epilepsy severity was measured using

the Hague Seizure Severity Scale (HASS) questionnaire, which parents completed with guidance from trained staff to ensure proper understanding of the questions.

In addition, the levels of vitamin D 25-OH, IL-1β, and GDNF were measured by collecting 4 cc of blood samples from the cubital vein. The serum was then transferred into individual cryotubes designated for each biomarker analysis. Each cryotube was labeled with a unique code corresponding to the participant's group and stored at -80°C until analysis. The levels of vitamin D 25-OH, IL-1β, and GDNF were measured using Elecsys Vitamin D total III (Roche Diagnostics GmbH, Mannheim, Germany), Human Interleukin 1β, IL-1β BT-LAB Kit (Bioassay Technology Laboratory; Jiaxing, China), and Human Glial Cell Line-derived Neurotrophic Factor, GDNF BT-LAB Kit (Bioassay Technology Laboratory; Jiaxing, China), respectively, following the manufacturer's recommendation.

Study variables

Independent variables in this study were vitamin D 25-OH, IL-1β, and GDNF levels. All of these variables were measured using ELISA. Vitamin D 25-OH levels were classified into two categories: normal (31–100 ng/mL) and abnormal (≤30 ng/mL). All measurements were conducted at the accredited laboratory of Adam Malik Hospital, Medan, Indonesia, to ensure the validity and reliability of the results. In addition, age and sex were also treated as independent variables.

The dependent variables in this study were seizure frequency and epilepsy severity. Seizure frequency was assessed using the GASE questionnaire. The GASE evaluates seven clinical aspects on a seven-point Likert scale, including seizure frequency, seizure intensity, injuries or falls during seizures, duration or severity of the postictal period, total dosage or number of antiepileptic drugs, side effects of antiepileptic drugs, and the impact of epilepsy or medications on activities of daily living. Scores range from 1 (not at all severe/absent/never) to 7 (very severe/extreme/very frequent/high) [25,26]. In this study, seizure frequency was measured using one aspect of the GASE only and scored from 1 (never) to 7 (very frequent). For analysis, the seizure frequency was categorized into two levels: non-frequent (1–4) and frequent (5–7).

Epilepsy severity was measured using the HASS questionnaire, which serves as a measure of perceived seizure severity by parents and is valuable as an outcome measure in pediatric epilepsy [27]. The HASS questionnaire evaluates 13 potential ictal and postictal problems experienced over the past three months [28]. The HASS includes questions addressing consciousness (four questions), motor symptoms (two questions), incontinence (one question), injury/pain (three questions), and severity (three questions). Ictal symptoms are represented in nine questions, while postictal symptoms are covered in four. The possible responses ranged from 1 (most favorable) to 4–5 (most unfavorable). Total scores range from 13 (lowest seizure severity) to 54 (highest seizure severity) [27]. The HASS scores were categorized into two levels: mild (scores 13–32) and severe (scores 33–52).

Statistical analysis

Normality tests for all variables were conducted using the Kolmogorov–Smirnov test. Correlation analysis was performed with the Pearson test for normally distributed data and the Spearman rank test for non-normally distributed data. The relationships between biomarker levels (vitamin D 25-OH, IL-1β, and GDNF level), seizure frequency (both per month and measured by GASE), and epilepsy severity (HASS score) were measured using multiple linear regression. Before regression analysis, regression assumptions—multicollinearity, heteroscedasticity, and linearity—were assessed to ensure model validity. A $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS software version 24.0 (IBM SPSS, Chicago, Illinois, USA).

Results

Characteristics of patients

In the present study, 73 children with drug-resistant epilepsy were included, and their characteristics are presented in **Table 1**. Out of the total, 40 were boys (54.8%), 31 children

(42.5%) were aged under 10 years old, and 42 adolescents (57.5%) were aged between 10 and 18 years old. The mean HASS score was 32.49±9.27, indicating a balanced distribution between mild and severe epilepsy (47.9% vs 52.1%). Vitamin D 25-OH level was found abnormal in 57 samples (78.1%). A total of 36 (49.1%) and 37 (50.9%) patients were classified as having non-frequent and frequent seizures based on the GASE questionnaire, with a median of five seizures per month. The median levels of IL-1β and GDNF were 284.42 pg/mL and 2,260 pg/mL, respectively (**Table 1**).

Table 1. Characteristics of the included patients (n=73)

Characteristics	Frequency (%)
Sex	
Male	40 (54.8)
Female	33 (45.2)
Age	
Children	31 (42.5)
Adolescent	42 (57.5)
Hague Seizure Severity Scale (HASS) score, mean±SD	32.49±9.27
Mild	35 (47.9)
Severe	38 (52.1)
Vitamin D 25-OH level (ng/mL), mean±SD	25.07±9.82
Normal (31–100 ng/mL)	16 (21.9)
Abnormal (≤30 ng/mL)	57 (78.1)
Seizure frequency	
Non-frequent	36 (49.1)
Frequent	37 (50.9)
Seizure frequency (month), median (min-max)	5 (1–244)
Interleukin-1β (IL-1β) level (pg/mL), median (min-max)	284.42 (29.24–2,967.66)
GDNF (pg/mL), median (min-max)	2,260.00 (30.00–11,490.00)

GDNF: glial cell-line derived neurotrophic factor

Relationship between vitamin D 25-OH, IL-1β and GDNF levels with epilepsy severity

GDNF levels had a significant association with epilepsy severity (p=0.036). Patients with higher GDNF levels tended to have higher HASS scores, indicating more severe epilepsy (**Table 2**). However, no significant association was found between vitamin D 25-OH or IL-1β levels and epilepsy severity (p=0.691 and p=0.838, respectively). These findings suggest that vitamin D 25-OH and IL-1β may not play a direct role in epilepsy severity among children with drug-resistant epilepsy (**Table 2**).

Table 2. Factors associated with epilepsy severity based on the Hague Seizure Severity Scale (HASS) score among children with drug-resistant epilepsy

Variable	Hague Seizure Severity Scale score		p-value
	Mild, n (%)	Severe, n (%)	
Sex			
Male	22 (30.1)	18 (24.6)	0.184[a]
Female	13 (17.8)	20 (27.4)	
Age			
Children	18 (24.6)	13 (17.8)	0.558[a]
Adolescent	17 (23.2)	25 (34.2)	
Vitamin D 25-OH level (ng/mL), mean±SD	25.50±9.89	24.62±9.87	0.691[b]
Normal	8 (10.96)	8 (10.96)	0.852[a]
Abnormal	27 (36.99)	20.00 (27.40)	
IL-1β level (pg/mL), median (min-max)	281.00 (29.24–1479.00)	289.91 (81.37–2967.66)	0.838[b]
GDNF level (pg/mL), median (min-max)	1370.00 (30.00–8160.00)	3315.00 (40.00–11490.00)	0.036[b*]

GDNF: Glial-cell-line derived neurotrophic factor; IL-1β: interleukin-1β
[a] Analyzed using Chi-squared test
[b] Analyzed using Mann-Whitney test
[*] Statistically significant at p<0.05

Relationship between vitamin D 25-OH, IL-1β levels, GDNF levels, and seizure frequency

Our data indicated that there was no significant association between vitamin D 25-OH, IL-1β, and GDNF levels and seizure frequency, as measured by the GASE scores ($p>0.05$ for all variables) (**Table 3**). This finding suggests that these factors do not directly influence seizure frequency among the patients with drug-resistant epilepsy in this study.

Table 3. Factors associated with seizure frequency among children with drug-resistant epilepsy

Variable	Seizure frequency		*p*-value
	Non-frequent, n (%)	Frequent, n (%)	
Sex			
Male	29 (39.73)	11 (15.07)	0.801[a]
Female	23 (31.51)	10 (13.70)	
Age			
Children	19 (26.03)	12 (16.44)	0.124[a]
Adolescent	33 (45.21)	9 (12.33)	
Vitamin D 25-OH level (ng/mL), mean±SD	26.71±11.44	24.40±9.13	0.368[b]
Normal	12 (16.44)	4 (5.48)	0.706[a]
Abnormal	40 (54.79)	17 (23.29)	
Interleukin-1β (IL-1β) level (pg/mL), median (min-max)	289.91 (29.24–2967.66)	279.85 (81.98–1479.00)	0.884[c]
GDNF level (pg/mL), median (min-max)	1895.00 (30.00–11490.00)	2970.00 (40–6760)	0.903[c]

GDNF: Glial-cell-line derived neurotrophic factor
[a] Analyzed by using Chi-squared test
[b] Analyzed by using ANOVA test
[c] Analyzed by using Mann-Whitney test

Correlation between vitamin D 25-OH, IL-1β, GDNF, seizure frequency, and HASS score

Correlations between vitamin D 25-OH, IL-1β, GDNF, seizure frequency, and HASS score are presented in **Table 4**. Vitamin D 25-OH levels did not significantly correlate with IL-1β levels, GDNF levels, seizure frequency per month, or epilepsy severity as measured by the HASS score. IL-1β levels correlated significantly only with GDNF levels ($r=0.525$, $p=0.001$). GDNF levels had a significant correlation with HASS scores ($r=0.318$, $p=0.006$), suggesting that increased GDNF levels are associated with greater epilepsy severity. However, GDNF levels did not correlate significantly with seizure frequency per month. Seizure frequency showed no significant correlations with any biomarkers or HASS scores.

Table 4. Correlations between vitamin D 25-OH, IL1β, GDNF, seizure frequency, and Hague Seizure Severity Scale (HASS) score

Parameters	Correlation coefficient and *p*-value	Vitamin D 25-OH level	IL-1β level	GDNF level	Seizure frequency	HASS score
Vitamin D 25-OH level	*r*	1.000	0.078	0.152	0.061	-0.055
	p-value		0.514[b]	0.198[b]	0.610[b]	0.644[a]
IL-1β level	*r*		1.000	0.525	-0.081	0.037
	p-value			0.001[b*]	0.496[b]	0.755[b]
GDNF level	*r*			1.000	-0.084	0.318
	p-value				0.481[b]	0.006[b*]
Seizure frequency	*r*				1.000	-0.058
	p-value					0.629[b]
HASS score	*r*					1.000
	p-value					

GDNF: Glial-cell-line derived neurotrophic factor; IL-1β: interleukin-1β
[a] Analyzed using Pearson correlation test
[b] Analyzed using Spearman correlation test
[*] Statistically significant at $p<0.05$

Discussion

In the present study, the relationship between various biomarkers—vitamin D 25-OH, IL-1β, and GDNF—and epilepsy severity and seizure frequency were investigated in children with drug-resistant epilepsy. The results indicated that vitamin D 25-OH levels did not show a significant association with either epilepsy severity or seizure frequency. This finding aligns with previous studies suggesting that, despite its neuroprotective role, the relationship between vitamin D 25-OH and epilepsy remains inconsistent [29-32]. Vitamin D 25-OH levels contribute to calcium homeostasis and neuronal function, with a link to mean seizure control observed clinically, although the underlying mechanism remains unclear [29-32]. Conversely, a study found that serum vitamin D 25-OH levels were associated with improved seizure control in some patients who were previously vitamin D deficient, although this effect was not universal for all patients [33]. Collectively, these studies suggest that the effects of vitamin D may be individualized and influenced by factors such as genetics, seizure type, or interactions with anti-seizure medications [34,35]. Nonetheless, the role of vitamin D as a neuroprotective agent remains significant and severe vitamin D deficiency should be prevented in epilepsy patients to avoid complications that may adversely affect their quality of life [29,36].

IL-1β is a proinflammatory cytokine involved in the pathogenesis of neurological diseases, including epilepsy [37]. In the present study, IL-1β levels were not significantly associated with epilepsy severity or seizure frequency; however, a positive correlation with GDNF levels was observed. This suggests that IL-1β may be more related to general inflammatory processes rather than directly influencing epilepsy severity [38-40]. IL-1β can trigger brain inflammation that contributes to epileptogenesis, although its correlation with seizure intensity and epilepsy severity remains inconsistent [38]. Furthermore, IL-1β may increase blood-brain barrier permeability, thereby heightening seizure sensitivity [38]. Other studies indicated that IL-1β may enhance blood-brain barrier permeability, thereby increasing seizure sensitivity [41,42]. A study found that IL-1β associated with the risk of developing drug-resistant epilepsy in pediatric patients, underscoring its role in epileptogenesis [43]. The relationship between IL-1β and GDNF in the present study may indicate a complex interaction between inflammatory and neurotrophic pathways, suggesting that further research is necessary to clarify the mechanisms underlying epilepsy.

GDNF levels had a significant relationship with epilepsy severity in the present study, as measured by the HASS score. As a neurotrophic factor, GDNF is crucial for neuronal survival, nerve regeneration, and overall neurological function [24]. The findings of the present study align with previous research, suggesting that increased GDNF levels may indicate a compensatory response to neurological damage in conditions such as epilepsy [44-46]. A study demonstrated GDNF's therapeutic potential in protecting neurons, which may explain the higher levels observed in patients with less severe epilepsy; however, this correlation is indirect and requires further investigation [47]. Additionally, GDNF has been shown to reduce seizure severity in animal models, supporting the idea that increased levels may reflect the body's attempt to mitigate neuronal damage from recurrent seizures [48,49]. Nevertheless, elevated GDNF levels may also indicate ongoing pathological processes [48,50]. A study found that long-term increases in GDNF could lead to maladaptive changes in brain circuitry, potentially worsening epilepsy; thus, the role of GDNF in epilepsy is complex and context-dependent [51]. While the present study suggests a positive association between GDNF and epilepsy severity, further research is needed to clarify its clinical implications and evaluate GDNF as a potential therapeutic target for reducing epilepsy severity.

Seizure frequency per month is a critical variable for assessing seizure control in epilepsy patients [52,53]. However, in the present study, no significant correlation was found between seizure frequency and levels of vitamin D 25-OH, IL-1β, GDNF, or HASS score. These findings suggest that seizure frequency may not accurately reflect the true severity of epilepsy or associated inflammatory and neurotrophic responses, especially in cases with treatment side effects or complications [33,52,54-56]. Consequently, while seizure frequency is commonly used as a clinical marker, the present study indicates that a more comprehensive assessment approach may be necessary to fully understand the impact of epilepsy on patients.

HASS score, used to assess epilepsy severity in the present study, exhibited a significant positive correlation with GDNF levels, but not with vitamin D 25-OH, IL-1β, or seizure frequency. HASS score encompasses various aspects of epilepsy severity, including seizure frequency, duration, and their impact on daily life [16,57,58]. These findings support the effectiveness of multidimensional approaches, such as the HASS score, in assessing epilepsy severity over a single clinical or biochemical parameter. Previous studies highlighted that tools like HASS or the GASE better captured the complexities of epilepsy and its effects on quality of life [9,59]. The correlation between HASS scores and GDNF levels aligns with theories on neurotrophic responses that may influence epilepsy severity [60]. However, it is crucial to apply severity assessment tools, such as HASS, cautiously due to potential influences from subjective factors and individual variations [9]. Therefore, integrating HASS results with additional biomarkers and clinical data is essential for a comprehensive understanding of the patient's condition.

The major limitations of this study include its cross-sectional design, which limits conclusions regarding causality and the dynamics of biomarker changes over time. The study's reliance on a single-center, purposive sample may reduce generalizability, particularly as the sample only includes pediatric patients with drug-resistant epilepsy. Furthermore, the use of self-reported seizure severity scales by parents also introduces the potential for subjective bias, especially as perceptions of severity can vary. Finally, despite the study's valuable insights into GDNF, IL-1β, and vitamin D 25-OH levels, it lacks a comprehensive exploration of other potential biomarkers or genetic factors that may influence epilepsy outcomes, which could provide a more holistic understanding of the disease.

Conclusion

GDNF levels significantly correlated with epilepsy severity, as measured by the HASS score, suggesting that GDNF may serve as a potential biomarker for assessing epilepsy severity. In contrast, vitamin D 25-OH and IL-1β levels did not show significant associations with either epilepsy severity or seizure frequency, indicating that these factors may not directly influence the pathophysiological mechanisms of epilepsy. Additionally, seizure frequency did not correlate with the measured biomarkers, suggesting that epilepsy severity is not always reflected in seizure frequency. These findings warrant further research to investigate the role of GDNF as a potential neurotrophic factor in the pathophysiology of epilepsy and its possible application as a therapeutic target.

Ethics approval

Protocol of the present study was approved by the Ethical Committee for Health Research, Faculty of Medicine, Universitas Sumatera Utara (Approval number: 1194/KEPK/USU/2023) and Adam Malik Hospital, Indonesia (Approval number: LB02.02/XV.III.2.2.2/85/2024) and is registered on ClinicalTrials.gov (Identifier: NCT06053281).

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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References

1. Henning O, Lossius MI, Lima M, *et al.* Refractory epilepsy and nonadherence to drug treatment. Epilepsia Open 2019;4(4):618-623.
2. Golyala A, Kwan P. Drug development for refractory epilepsy: The past 25 years and beyond. Seizure 2017;44:147-156.
3. López GFJ, Rodríguez OX, Gil-Nagel RA, *et al.* Drug-resistant epilepsy: Definition and treatment alternatives. Neurol Engl Ed 2015;30(7):439-446.
4. Rosati A, De Masi S, Guerrini R. Antiepileptic drug treatment in children with epilepsy. CNS Drugs 2015;29(10):847-863.
5. Nasiri J, Ghazzavi M, Sedghi M, *et al.* Causes and risk factors of drug-resistant epilepsy in children. Iran J Child Neurol 2023;17(3):79-85.
6. Aggarwal HK, Jain D, Bishnoi A. Quality of life in patients with drug resistant epilepsy. Turk Noroloji Derg 2019;25(3):159-163.
7. Mula M, Cock HR. More than seizures: Improving the lives of people with refractory epilepsy. Eur J Neurol 2015;22(1):24-30.
8. Jain P, Subendran J, Smith M Lou, *et al.* Care-related quality of life in caregivers of children with drug-resistant epilepsy. J Neurol 2018;265(10):2221-2230.
9. Nagabushana D, S. PK, Agadi JB. Impact of epilepsy and antiepileptic drugs on health and quality of life in Indian children. Epilepsy Behav 2019;93:43-48.
10. Bazhanova ED, Kozlov AA, Litovchenko A V. Mechanisms of drug resistance in the pathogenesis of epilepsy: Role of neuroinflammation. A literature review. Brain Sci 2021;11(5):663.
11. Barker-Haliski ML, Löscher W, White HS, *et al.* Neuroinflammation in epileptogenesis: Insights and translational perspectives from new models of epilepsy. Epilepsia 2017;58:39-47.
12. Pracucci E, Pillai V, Lamers D, *et al.* Neuroinflammation: A signature or a cause of epilepsy? Int J Mol Sci 2021;22(13):1-18.
13. Mazarati AM, Lewis ML, Pittman QJ. Neurobehavioral comorbidities of epilepsy: Role of inflammation. Epilepsia 2017;58:48-56.
14. Uludag IF, Duksal T, Tiftikcioglu BI, *et al.* IL-1β, IL-6 and IL1Ra levels in temporal lobe epilepsy. Seizure 2015;26:22-25.
15. Cortés D, Carballo-Molina OA, Castellanos-Montiel MJ, *et al.* The non-survival effects of glial cell line-derived neurotrophic factor on neural cells. Front Mol Neurosci 2017;10:258.
16. Shpak AA, Rider FK, Druzhkova TA, *et al.* Reduced levels of lacrimal glial cell line-derived neurotrophic factor (GDNF) in patients with focal epilepsy and focal epilepsy with comorbid depression: A biomarker candidate. Int J Mol Sci 2023;24(23):16818.
17. Bivona G, Gambino CM, Iacolino G, *et al.* Vitamin D and the nervous system. Neurol Res 2019;41(9):827-835.
18. Mpandzou G, Aït Ben Haddou E, Regragui W, *et al.* Vitamin D deficiency and its role in neurological conditions: A review. Rev Neurol 2016;172(2):109-122.
19. Filgueiras MS, Rocha NP, Novaes JF, *et al.* Vitamin D status, oxidative stress, and inflammation in children and adolescents: A systematic review. Crit Rev Food Sci Nutr 2020;60(4):660-669.
20. Mousa A, Misso M, Teede H, *et al.* Effect of vitamin D supplementation on inflammation: Protocol for a systematic review. BMJ Open 2016;6(4):1-5.
21. Miratashi YSA, Abbasi M, Miratashi YSM. Epilepsy and vitamin D: A comprehensive review of current knowledge. Rev Neurosci 2017;28(2):185-201.
22. Nagarjunakonda S, Amalakanti S, Uppala V, *et al.* Vitamin D in epilepsy: Vitamin D levels in epilepsy patients, patients on antiepileptic drug polytherapy and drug-resistant epilepsy sufferers. Eur J Clin Nutr 2016;70(1):140-142.
23. Zhang S, Chen F, Zhai F, *et al.* Role of HMGB1/TLR4 and IL-1β/IL-1R1 signaling pathways in epilepsy. Front Neurol 2022;13(6):1-11.
24. Kotliarova A, Sidorova YA. Glial cell line-derived neurotrophic factor family ligands, players at the interface of neuroinflammation and neuroprotection: Focus onto the glia. Front Cell Neurosci 2021;15:679034.

25. Speechley KN, Sang X, Levin S, *et al.* Assessing severity of epilepsy in children: Preliminary evidence of validity and reliability of a single-item scale. Epilepsy Behav 2008;13(2):337-342.
26. Chan CJ, Zou G, Wiebe S, *et al.* Global assessment of the severity of epilepsy (GASE) Scale in children: Validity, reliability, responsiveness. Epilepsia 2015;56(12):1950-1956.
27. Carpay JA, Vermuelen J, Stroink H, *et al.* Seizure severity in children with epilepsy: A parent-completed scale compared with clinical data. Epilepsia 1997;38(3):346-352.
28. Carpay H. Childhood epilepsy: Alternative strategies for assessing treatment strategies and outcome (Thesis). Tijdschr Kindergeneeskd 1998;66(2):96.
29. Chassoux F, Navarro V, Quirins M, *et al.* Vitamin D deficiency and effect of treatment on seizure frequency and quality of life parameters in patients with drug-resistant epilepsy: A randomized clinical trial. Epilepsia 2024(6):1-14.
30. Anjum I, Jaffery SS, Fayyaz M, *et al.* The role of vitamin D in brain health: A mini literature review. Cureus 2018;10(7):e2960.
31. Mayne PE, Burne THJ. Vitamin D in synaptic plasticity, cognitive function, and neuropsychiatric illness. Trends Neurosci 2019;42(4):293-306.
32. Cui X, Gooch H, Groves NJ, *et al.* Vitamin D and the brain: Key questions for future research. J Steroid Biochem Mol Biol 2015;148:305-309.
33. Alhaidari HM, Babtain F, Alqadi K, *et al.* Association between serum vitamin D levels and age in patients with epilepsy: A retrospective study from an epilepsy center in Saudi Arabia. Ann Saudi Med 2022;42(4):262-268.
34. Kong AN, Fong CY, Ng CC, *et al.* Association of common genetic variants with vitamin D status in Malaysian children with epilepsy. Seizure 2020;79(4):103-111.
35. Jiang P, Zhu WY, He X, *et al.* Association between vitamin D receptor gene polymorphisms with childhood temporal lobe epilepsy. Int J Environ Res Public Health 2015;12(11):13913-13922.
36. Anwar MJ, Alenezi SK, Alhowail AH. Molecular insights into the pathogenic impact of vitamin D deficiency in neurological disorders. Biomed Pharmacother 2023;162(4):114718.
37. Kamaşak T, Dilber B, Yaman SÖ, *et al.* HMGB-1, TLR4, IL-1R1, TNF-α, and IL-1β: Novel epilepsy markers? Epileptic Disord 2020;22(2):183-193.
38. Vezzani A, Balosso S, Ravizza T. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. Nat Rev Neurol 2019;15(8):459-472.
39. de Vries EE, van den Munckhof B, Braun KPJ, *et al.* Inflammatory mediators in human epilepsy: A systematic review and meta-analysis. Neurosci Biobehav Rev 2016;63:177-190.
40. Sun Y, Ma J, Li D, *et al.* Interleukin-10 inhibits interleukin-1β production and inflammasome activation of microglia in epileptic seizures. J Neuroinflammation 2019;16(1):1-13.
41. Swissa E, Serlin Y, Vazana U, *et al.* Blood–brain barrier dysfunction in status epileptics: Mechanisms and role in epileptogenesis. Epilepsy Behav 2019;101:106285.
42. Löscher W. Epilepsy and alterations of the blood–brain barrier: Cause or consequence of epileptic seizures or both? Handb Exp Pharmacol 2022;273:331-350.
43. Choi J, Kim SY, Kim H, *et al.* Serum α-synuclein and IL-1β are increased and correlated with measures of disease severity in children with epilepsy: Potential prognostic biomarkers? BMC Neurol 2020;20(1):1-11.
44. Ibáñez CF, Andressoo JO. Biology of GDNF and its receptors — Relevance for disorders of the central nervous system. Neurobiol Dis 2017;97:80-89.
45. Cintrón-Colón AF, Almeida-Alves G, Boynton AM, *et al.* GDNF synthesis, signaling, and retrograde transport in motor neurons. Cell Tissue Res 2020;382(1):47-56.
46. Azevedo MD, Sander S, Tenenbaum L. GDNF, a neuron-derived factor upregulated in glial cells during disease. J Clin Med 2020;9(2):456.
47. Airaksinen MS, Saarma M. The GDNF family: Signalling, biological functions and therapeutic value. Nat Rev Neurosci 2002;3(5):383-394.
48. Kustova AO, Gavrish MS, Sergeeva MA, *et al.* The Influence of Neurotrophic Factors BDNF and GDNF Overexpression on the Functional State of Mice and Their Adaptation to Audiogenic Seizures. Brain Sci 2022;12(8):1039.
49. Chiavellini P, Canatelli-Mallat M, Lehmann M, *et al.* Therapeutic potential of glial cell line-derived neurotrophic factor and cell reprogramming for hippocampal-related neurological disorders. Neural Regen Res 2022;17(3):469-476.
50. Mikroulis A, Waloschková E, Bengzon J, *et al.* GDNF increases inhibitory synaptic drive on principal neurons in the hippocampus via activation of the ret pathway. Int J Mol Sci 2022;23(21):1-22.

51. Singh G, Sikder A, Phatale V, *et al.* Therapeutic potential of GDNF in neuroinflammation: Targeted delivery approaches for precision treatment in neurological diseases. J Drug Deliv Sci Technol 2023;87(6):104876.
52. Teagarden DL, Meador KJ, Loring DW. Low vitamin D levels are common in patients with epilepsy. Epilepsy Res 2014;108(8):1352-1356.
53. Geremew GW, Wassie YA, Tadesse G, *et al.* Treatment outcome and its predictors among children with epilepsy on chronic follow-up in Ethiopia: A systematic review and meta-analysis. BMC Public Health 2024;24(1):3001.
54. Tombini M, Assenza G, Quintiliani L, *et al.* Epilepsy and quality of life: What does really matter? Neurol Sci 2021;42(9):3757-3765.
55. Holló A, Clemens Z, Kamondi A, *et al.* Correction of vitamin D deficiency improves seizure control in epilepsy: A pilot study. Epilepsy Behav 2012;24(1):131-133.
56. Tombini M, Palermo A, Assenza G, *et al.* Calcium metabolism serum markers in adult patients with epilepsy and the effect of vitamin D supplementation on seizure control. Seizure 2018;58:75-81.
57. Paolone G, Falcicchia C, Lovisari F, *et al.* Long-Term, Targeted Delivery of GDNF from Encapsulated Cells Is Neuroprotective and Reduces Seizures in the Pilocarpine Model of Epilepsy. J Neurosci 2019;39(11):2144-2156.
58. He G, Zhu J, Li B. Parent/caregiver reported health-related functioning in Chinese children with epilepsy: A cross-sectional, parents-responded, hospital-based study. Medicine 2023;102(12):e33168.
59. Duarte EP, Corrêa CD, Marques BS, *et al.* Severity and disability related to epilepsy from the perspective of patients and physicians: A cross-cultural adaptation of the GASE and GAD scales. Seizure 2021;90(11):93-98.
60. Wang X, Hu Z, Zhong K. The role of brain-derived neurotrophic factor in epileptogenesis: An update. Front Pharmacol 2021;12(11):1-7.