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The Effect of Vitamin D Supplementation on Changes in TNF-α Levels and Exciting Frequency in Epilepsy Patients

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ABSTRACT

Background: Epilepsy, a neurological disorder, is a global public health problem. The active form of vitamin-D is 5000IU/day for 42 days, able to push inflammation and change the balance between inhibitory cytokine and excitatory cytokine, to suppress recurrent seizures.
Objective: Analyze the effect of vitamin-D 5000 supplementations on changes in TNF-α levels pre-and-post-vitamin-D supplementation and changes in the frequency of seizures pre-and post-vitamin-D supplementation in epilepsy patients.
Methods: : This research is a quasi-experimental analytic observational study with a pre-and-post-tests approach without control. Subjects were patients diagnosed with epilepsy who had met the inclusion criteria. Previous research subjects were checked for TNF-α levels, then given vitamin-D supplementation of 5000IU/day for 42 days, then rechecked TNF-α levels. Then performed a bivariate analysis.
Results: There was a significant difference between changes in TNF-α pre-and-post-levels of vitamin-D supplementation of 5000IU/day for 42 days ($p<0.001$). There was a significant difference in the frequency of seizures pre-and-post-levels of vitamin D supplementation 5000IU/day for 42 days ($p=0.002$). There is a significant relationship between (δ)TNF-α on (δ)the frequency of seizures pre-and-post-vitamin-D supplementation 5000IU/day, strongly and positively related ($p<0.001$; $r=0.661$). There is no relationship between risk factors with (δ)TNF-α. There is no relationship between risk factors with (δ)seizure frequency.
Conclusion: There is a significant difference in the levels of TNF-α pre-and-post-vitamin-D supplementation. There is a significant difference between the frequency of seizures pre-and-post-vitamin-D supplementation. There is a relationship between (δ)TNF-α and (δ)the frequency of seizures pre-and-post-vitamin-D supplementation.

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1. Introduction

Epilepsy is a neurological disorder that has become a global public health problem.[1] Treatment of epilepsy with antiepileptic drugs (AED) is a long-term treatment and often involves several drugs so we must pay attention to the side effects of each AED.[2] One of the side effects that have become a concern of clinicians recently is vitamin D deficiency. The prevalence of vitamin D deficiency is increasing every year, this number is increasing in people with epilepsy.[3] Research shows that the active form of vitamin D suppresses inflammation and alters the balance between inhibitory and excitatory cytokines.[4] Inflammatory reactions occur in the brain in a variety of central nervous system diseases, including autoimmune, neurodegenerative, and epilepsy disorders.

Experimental studies in rodents have shown that inflammatory reactions in the brain can increase nerve excitability, interfere with cell survival, and increase the permeability of the blood-brain barrier to blood-borne molecules and cells. In addition, some anti-inflammatory medications reduce seizures in experimental models and some cases, in clinical epilepsy cases.[5,6] The pro-inflammatory cytokine TNF-α is expressed and activated by microglia and astrocytes.

Glial cells have a function to detect extracellular glutamate levels, and if glutamate is detected low, glial cells will stimulate TNF-α to upregulate synapses and maintain a certain level of neuronal excitation input. TNF-α has been found to increase glutamate release in microglia through the upregulation of glutaminase and increased glutamate levels in the inter-synaptic cleft. In addition, TNF-α also upregulates AMPA receptors and increases glutaminergic transmission.

Increased AMPA receptors will allow the absorption of calcium (Ca^{2+}) in excess which will cause neurotoxicity. TNF-α not only stimulates an increase in glutamate but also induces GABA receptor endocytosis, reducing the inhibitory process and causing an imbalance between excitation and inhibition, which will allow for overexcitation.[4] This study aimed to analyze the effect of Vitamin D supplementation on changes in TNF-α levels pre- and post-vitamin D supplementation and changes in the

frequency of seizures pre- and post-vitamin D supplementation in epilepsy patients.

2. Methods

This study is experimental with a pre and post-test without control design. This research was conducted at RSUP Dr. Kariadi Semarang, which was held from May 2022 to June 2022. The research sample was from epilepsy patients in the outpatient department of Neurology, Dr. Kariadi Semarang. The sampling method used is consecutive sampling. Inclusion criteria included patients diagnosed with primary and secondary epilepsy, patients who have used anti-epileptic drug (AED) therapy for more than 1 year, patients in category of teenager (11-19 years) and adult (20-60 years) according to WHO classification, and patients/families who agreed to participate in the study by signing informed consent. Exclusion criteria included patients with acute symptomatic seizures, patients with BMI < 18.50 kg/m2, patients with impaired renal function, and patients with impaired liver function. Dropout criteria included study subjects who did not use supplementation for 42 days and patients during the study process who experienced diarrhea associated with impaired absorption of vitamin D supplementation.

Research subjects receive vitamin D supplementation at a dose of 5000 IU/day for 42 days. Measurements were obtained before and after supplementation, including TNF-α levels from venous blood sample and seizure frequency. Additional data collected included AED adherence assessed using the MMAS-8 (Morisky Adherence Scale) categorized as high or moderate adherence, and sun exposure, classified as adequate and inadequate based on a threshold of 1 minimum erythemal dose (MED) per hour (25 minute from 09.00 – 12.00, three times per week)

The data that has been collected was tested using Paired T-test, Wilcoxon test, and contingency coefficient test.

This research has obtained permission from the Health Research Ethics Commission, Faculty of Medicine, University of Diponegoro / Dr. RSUP. Kariadi Semarang and the Medical Council of Dr. RSUP. Kariadi Semarang No.1000/EC/KEPK-RSDK/2022.

3. Result

Patients with epilepsy from May 2022 to June 2022 at RSUP Dr. Kariadi that meet the inclusion criteria and do not have the exclusion criteria totaling 33 people. The characteristics of the research subjects were obtained in table 1.

Table 1. Characteristics of research subjects.

Variable	Total	Changes in TNF-α levels		*P*	Changes in Seizure Frequency		*P*
		Increase	Decrease		Increase	Decrease	
Age • Teenager (11-19 years) • Adult (20-60 years)	8 (24.2) 25 (75.8)	6 15	2 10	0.443¥	4 15	4 10	0.618¥
Gender • Male • Female	13 (39.4) 20 (60.6)	8 13	5 7	0.840¥	7 12	6 8	0.727¥
AED Consumption Duration • 1-2 year • >2 years	3 (9.1) 30 (90.9)	2 19	1 11	0.909¥	2 17	1 13	0.738¥
AED Consumption Amount • Monotherapy • Polytherapy	7 (21.2) 26 (78.8)	6 15	1 11	0.171¥	5 14	2 12	0.403¥
AED Compliance • High • Moderate	29 (87.9) 4 (12.1)	18 3	11 1	0.614¥	16 3	13 1	0.452¥
Sun Exposure • Adequate • Inadequate	30 (90.9) 3 (9.1)	18 3	12 0	0.170¥	16 3	14 0	0.119¥

¥Contingency coefficient test

In the assessment of 33 research subjects, most of the subjects were adults (75.8%) which were dominated by women (60.6%). Based on the use of antiepileptic drugs, most of the subjects had taken AED $\geq$ 2 years (90.9%) with the number of AED namely polytherapy (78.8%) with a high level of adherence (87.9%). Most of the subjects had a history of adequate sun exposure (90.9%).

After being given vitamin D supplementation of 5000 IU/day for 42 days, the subjects were assessed for increased or decreased levels of TNF-α and the frequency of seizures. There was no relationship between changes in TNF-α levels with age (p=0.443), gender (p=0.840), duration of AED consumption (p=0.909), number of AED (p=0.171), AED compliance (p=0.614), and a history of sun exposure (p=0.170). There was no correlation between changes in seizure frequency with age (p=0.618), gender (p=0.727), duration of AED consumption (p=0.738), number of AED (p=0.403), AED compliance (p=0.452), and history of sun exposure (p=0.119).

There is no significant relationship between risk factors and changes in (δ) levels of TNF-α. Age (p = 0.443), gender (p = 0.840), duration of antiepileptic drug treatment (p = 0.909), number of antiepileptic drugs (p = 0.171), adherence to taking antiepileptic drugs (p = 0.614), and exposure to sunlight (p = 0.170). This is following the research of Kobylarek D et al. who stated that neuroinflammation is a major factor in epileptogenesis. This neuroinflammation greatly affects the inflammatory mediator, namely TNF-α. Many hypotheses explain the etiopathogenesis underlying epilepsy, including neurodegeneration, disruption of the blood-brain barrier, amygdala dysregulation, changes in the glutamatergic system, oxidative stress, hypoxia, and epigenetic DNA modification. Most studies on inflammation and epilepsy have demonstrated the important role of inflammatory markers in epileptogenesis through the dysregulation of cytokine balance in the central nervous system.[7]

There is an insignificant relationship between risk factors and changes in (δ) seizure frequency. Age (p =

0.443), gender (p = 0.840), duration of antiepileptic drug treatment (p = 0.909), number of antiepileptic drugs (p = 0.171), adherence to taking antiepileptic drugs (p = 0.614), and exposure to sunlight (p = 0.170). This is following the study of Kobylarek D et al which stated that TNF-α is a prominent pro-inflammatory marker, some patients showed improved seizures after a decrease in TNF-α levels. In this study, patients experienced a reduction in seizure frequency. So changes in seizure frequency are influenced by changes in TNF-α levels.[7]

In this study, there was no relationship between risk factors and changes in (δ) TNF-α levels and no relationship between risk factors and changes in (δ) seizure frequency.

Table 2. TNF-α levels and seizure frequency

Variable	Mean ± SD	Median (min-max)	*P*
Gender • Pre • Post	156.53 ± 137.83 175.16 ± 130.11	139.7 (0.3-481.1) 170.3 (0.3-460.8)	<0.001†
Seizure Frequency • Pre • Post	8.66 ± 26.4 7.63 ± 26.5	2 (0-150) 1(0-150)	0.002‡

†Paired T-test; ‡Wilcoxon test

There was an increase in TNF-α levels between before and after vitamin D supplementation of 5000 IU/day with a significant difference in value (p<0.001). There was a decrease in the frequency of seizures between before and after vitamin D supplementation of 5000 IU/day with a significant difference in value (p=0.002).

In epilepsy, there is an imbalance in the regulation of activation and resolution of inflammatory cells. Sources and targets of the underlying irregular and overlapping inflammatory components in epileptogenesis.[4] Research conducted by Ghorbani Z, et al. Based on the results of this study, it was revealed that vitamin D3 supplementation can improve the balance of TNF-α related cytokines.[8] Based on the research of Groves NJ, et al., it was explained that vitamin D could suppress inflammation and change the balance between inhibitory and excitatory cytokines. Giving vitamin D will inhibit the production of TNF-α.[9] In addition, after vitamin D supplementation there was a decrease in the frequency of seizures. This is following the research of Hollo A, et al which stated that there was a significant reduction in the frequency of seizures after vitamin D supplementation.[10] Based on another study, DeGiorgio CM, et al., giving vitamin D3 supplementation (5000 IU/day), were able to reduce seizures from 5.18 seizures/month to 3.64 seizures/month for 6 weeks.[11]

Table 3. Correlation between changes in TNF-α levels and seizure frequency

Variable	Changes in Seizure Frequency		*P*	r
	Increase	Decrease		
Changes in TNF- α level • Increase • Decrease	 19 0	 2 12	<0.001¥	0.661

¥Contingency coefficient test; *significant p<0,05

There is a correlation between changes in TNF-α levels with changes in seizure frequency (p<0.001) with a strong and positive correlation level (r=0.661). This means that an increase in TNF-α levels correlates with an increase in the frequency of seizures and vice versa.

This is following the study of Kegler A et al. Based on the results of the study, the inflammatory marker TNF-α was significantly higher in the seizure-type epilepsy group compared to the control group.[12]

The limitations of this study include the absence of a comparative control group, a relatively short evaluation period, the lack of assessment of fluctuations in TNF- α levels over time, and the absence of standardized timing for nighttime vitamin D supplementation, which may have introduced bias. Therefore, future studies should adopt a longitudinal design using case control or cohort methodologies to allow extended follow up and comparision with baseline measurements at 6, 12, and 24 months or longer. Additionally subsequent research should incorporate pre and post intervention assessments with an appropriate control group to better evaluate correlations with relevant risk factors.

4. Conclusion

There is a relationship between (δ) TNF-α and (δ) the frequency of seizures pre- and post-vitamin-D supplementation.

Ethical Approval

Ethical clearance was secured from the Ethical Committee of the Faculty of Medicine, Diponegoro University No. 1000/EC/KEPK-RSDK/2022 prior to the commencement of the study.

Conflicts of Interest

There are no conflicts of interest to disclose.

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Author Contributions

Conceptualization, RD, EK, AH, R, HS, RA; methodology, RD, EK, AH, R, HS, RA; software, RD; validation, RD, EK, AH, R, HS, RA; formal analysis, EK, AH; investigation, RD; resources, RD; data curation, RD, EK, AH; writing— original draft preparation, RD, EK, AH,

R,HS, RA; writing—review and editing, RD, EK, AH, R, HS, RA; supervision, EK, AH; project administration, RD.

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