RESEARCH ARTICLE

Vitamin E level in epileptic patients attending Neuroscience hospital in Baghdad

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ABSTRACT

INTRODUCTION: Epilepsy is a highly prevalent and important neurological disease in the world. At least 50 million people worldwide are affected by the disease, and nearly 100 million people have experienced a seizure at least once in their lives.

Vitamin E deficiency has been reported in patients with epilepsy, though its clinical significance remains uncertain. This deficiency has been attributed to anti-epileptic therapy.

OBJECTIVE: To assess the level of vitamin E in epileptic patients attending the epilepsy clinic at the Neuroscience hospital in Baghdad in 2017 compared to non-epileptic patients. And to evaluate the association of vitamin E levels with age, gender, types of epilepsy and treatment used.

METHODS: A case-control study was conducted at the Epilepsy clinic in the Neuroscience hospital during the period between April 2017 and July 2017. Fifty patients diagnosed with unprovoked seizures of at least two attacks were included in the study. Vitamin E level was measured for each patient, The mean level of vitamin E of the patients with epilepsy was compared to that of the healthy control group. We studied the association of the mean level of vitamin E with the type of epilepsy, its duration, medicines used and their duration, and resistance to anti-epileptics.

RESULTS: The study found that 60% of the patients were males. Focal epilepsy was reported in 70% of the patients and generalized epilepsy in 30 %. Patients on one anti-epileptic were 40%, on two were 36% and on more than two were 20%. The mean level of vitamin E in patients with epilepsy was 4.3±0.79 µg/dl compared to 11.03±4.08 µg/dl (P>0.0001). Although the mean level of vitamin E is lower in patients with generalized epilepsy than focal, in those using enzyme inducer than those using non-enzyme inducer anti-epileptics, in those with drug resistance than those using drug response, and in those using more than two anti-epileptics than those using two or less; however these differences shows no statistically significant association, with p-values of 0.79, 0.76, 0.79, and 0.62, respectively.

CONCLUSION: The mean level of vitamin E vitamin is lower in patients with epilepsy than in those without. Type of epilepsy, type and the number of anti-epileptics used, and their resistant status showed no statistically significant association.

Key words: Epilepsy, vitamin E, focal, generalized.

INTRODUCTION

Epilepsy is a highly prevalent and important neurological disease in the world. At least 50 million people worldwide are affected by the disease, and nearly 100 million have experienced a seizure at least once. The disease creates psychological, physical, social, and economic consequences. Its prevalence is 5.8/1000 in developed countries and 10.3/1000 people in developing countries.[1]

The most common drugs used to treat epilepsy are valproate (VPA) sodium, carbamazepine, phenytoin, phenobarbital, lamotrigine and recently levetiracetam, usually in combination. Choosing any of these drugs depends on the type of seizure and the involved part of the brain. One of the drawbacks of carbamazepine, sodium VPA, and levetiracetam is the impact on the course of oxidative stress.[1,2,3]

In recent years, numerous antiepileptic drugs (AEDs) have been developed, but several unmet clinical needs remain, including resist-

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ance to AEDs found in about 30% of patients, adverse effects elicited by AEDs that can further reduce the quality of life, and the lack of treatments that can prevent the development of epilepsy in patients at risk .[5]

Oxidative stress is mitochondrial dysfunction and an imbalance between pro-oxidant and antioxidants that is involved in many pathological and physiological processes of many neurodegenerative disorders such as epilepsy have been reported. Brain cells are sensitive to reactive oxygen types produced during oxidation.[6] Oxidative stress can cause irreversible damage to biological molecules such as proteins, lipids, carbohydrates, and DNA. [7,8,9,10]

Vitamin E was discovered in 1922 by Evans and Bishop,[11] and it is a natural antioxidant[12] acting as a peroxyl radical scavenger and chain breaker of lipid peroxidation.[13] Eight forms of this vitamin have been identified as relevant for human and animal nutrition that are categorized into tocopherols (T) and tocotrienols (T3), with the latter bearing an unsaturated side chain condensed in position two of a chroman ring. Each of these two subfamilies is further categorized as α-, β-, γ - or δ-forms, which are defined by the number and location of methyl groups on the chromanol ring. The hydroxy group in position six of the chroman ring of vitamin E is the active site involved in the H atom donation effect essential for the chain-breaking (anti-peroxidase) activity of this vitamin. [14] Whereas the side chain is involved in the docking of vitamers in the lipid structure of cell membranes and lipoproteins. Among the vitamin E isomers, RRR-α-T has the highest in vivo bioavailability and bioactivity tested with different protocols, and also shows the highest H-donating activity in vitro.[15]

Vitamin E deficiency has been reported in patients with epilepsy, though its clinical significance remains uncertain. This deficiency has been attributed to antiepileptic therapy.[16] Vitamin E (or α–tocopherol/ alpha-tocopherol) prevents the damaging effects of oxidation in brain tissues. Free radical scavengers, such as vitamin E, have been implicated in decreasing prolonged seizure activity. Vitamin E is a natural nutrient that works to stabilize the membranes of cells and has no known toxic effects. Early animal studies, published in the Annals of Internal Medicine found that rats and mice that were exposed to 100% oxygen had seizures that can be prevented by giving vitamin E.[17]

In 1989 Ogunmekan et al conducted a study on children with epilepsy, and it was found that adding Vitamin E to AEDs reduces their seizures.[18] In another study conducted by Kovalenko et al, it was found that adding Vitamin E to anti-epileptic reduces plasma levels of lipid peroxidation and decreases the frequency of seizures in these patients.[19]

Aim of the study was to assess the level of vitamin E in epileptic patients attending the epilepsy clinic in Neuroscience Hospital in Baghdad 2017 in comparison with non-epileptic patients. And to evaluate the association of vitamin E levels with the management, gender, age and type of epilepsy categories.

METHODS

Study design and the setting: A cross-sectional study with analytic elements was conducted at the Epilepsy Clinic at the Neuroscience, SAAD Al-Witri, Hospital between the 1st of April 2017 and July 2017.

Ethical consideration: The study was conducted at the epilepsy clinic of SAAD Al-Witri Hospital after taking the agreement of the administration. Consent was taken from all participants before participation after explaining the objectives of the study.

Definition of the sample: All patients who visited the epilepsy clinic and were known to have two and more attacks of seizures without provoking factors like fever, hypoglycaemia, drugs, infection, recent trauma, etc were enrolled in the study. The diagnosis of seizure in those patients was made by consultant neurologists.

Sample size and the sampling: the sample was chosen conveniently from the targeted population. ***Comparison group***: for comparison a group of normal persons were selected conveniently from the attendants of the patients visiting the same hospital.

Study tool: The researchers tailored a questionnaire form to ensure proper data collection. All the forms were filled out by the researcher based on direct interviews with patients and their families.

Outcome and procedures: The outcome of the study was the level of vitamin E in the blood. Patients were grouped into generalized and focal epilepsy; on no, one, or two and above medications; and drug-resistant and drug-response patients. The level of vitamin E was compared among patients in these groups, in addition to those in the control group.

Drug-resistant epilepsy is defined as the failure of adequate trials of two tolerated and appropriately chosen drugs, single or in combination, to achieve sustained seizure freedom, using anti-epileptic drug schedules. Generalized epilepsy in this study includes generalized tonic-clonic seizures, myoclonic seizures, tonic, atonic and absence seizures, while the focal epilepsy includes simple partial, complex partial and secondary generalization.

Test principle and clinical relevance: The serum concentration of vitamin E is measured using high-performance liquid chromatography (HPLC) with photodiode array detection, a small volume (100 ml) of butyrate, and the micronutrients are extracted from the aqueous phase into hexane and dried under vacuum. The extract is dissolved in ethanol and acetonitrile and is filtered to remove insoluble material. An aliquot of the filtrate is injected into a c 18 reversed-phase column and isocratic ally eluted with a mobile phase consisting of equal parts of ethanol and acetonitrile. The absorbance of these substances in solution is linearly proportional to concentration (within limits), thus spectrophotometric methods are used for quantitative analysis. Three wavelengths approximately corresponding to absorption maxima 300,325, and 450 nm are simultaneously monitored and chromatograms are recorded. Quantitation is accomplished by comparing the peak height or area of the analyte in the unknown with the peak height or area of the unknown amount of the same analyte in a calibre solution. Calculations are corrected based on the peak height or area of the internal standard in the unknown compared with the peak height or area of the internal standard in a calibre solution. Retinol and retinyl butyrate at 325nm, tocopherol is compared with retinyl butyrate at 300 nm.

Statistical analysis: The data has been managed and analysed with computer software SPSS version 24. Data were shown as frequency tables and graphs. An Independent sample t-test was used to compare means in the variables of two categories. ANOVA test was used to compare means in the variables of more than two categories. A confidence level of 95% with a P-value equal to or less than 0.05 was considered significant.

RESULTS

Data were collected from the records of 50 patients with epilepsy attending the epilepsy clinic at the Neuroscience Hospital during the period between the 1st of April 2017 and July 2017.

The study found that 30 (60 %) of the patients were males and 20 (40%) were females. Focal epilepsy was found in 35 (70%) patients, and 15 (30%) had generalized epilepsy. Patients using one drug were 20 (40%), and two drugs 18 (36%), and more than two 10 (20%), see table 1. Figure 1 shows that two-thirds of the patients were drug-responded epilepsy while one-third was drug-resistant epilepsy.

Table 1 | Frequency distribution of the variables

Variable		No.	%
Gender	Male	66	60
	Female	44	40
Cases	Epileptic	50	45.5
	Non-epileptic	60	55.5
Type of epilepsy	Focal	35	70
	Generalized	15	30
Medication	Enzyme inducer	30	60
	Non-enzyme inducer	18	36
	No medication	2	4
Number of drugs	No drug	2	4
	One	20	40
	Two	18	36
	More than two	10	20

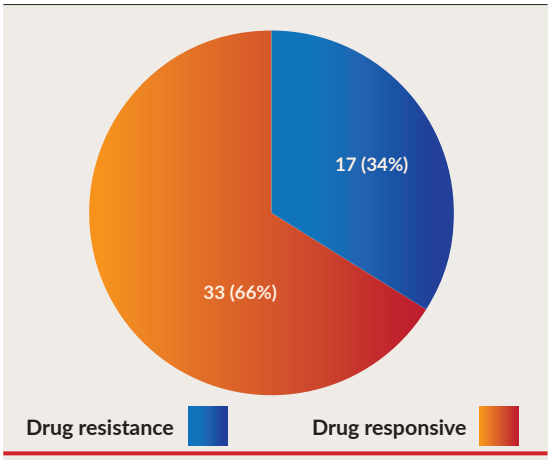

Figure 1 | Proportion of the drug resistance epilepsy (n 50).

Table 2 | Mean level of vitamin E in patients with epilepsy and in control.

Groups	Vitamin E level (µg/dl)		P-value
	Mean	±SD	
With Epilepsy (n 50)	4.30	0.79	t= 11.449
Without epilepsy (n 60)	11.03	4.08	P= 0.0001(*)

- Independent sample t test used to test the mean difference
* Statistically significant at alpha level of less than 0.05

Table 2 shows that the mean level of vitamin E level was 11.03±4.08 µg/dl in the non-epileptic group compared to 4.3±0.79 µg/dl in the group with epilepsy, this difference was statistically significant with a p-value of 0.0001.

Table 3 shows the association of vitamin E levels with the type of epilepsy, type and number of medications and drug resistance. No statistically significant difference was found between the mean level of vitamin E and focal and generalized epilepsy (P=0.79), the type (P=0.76) and the number of medications (P=0.62), and the presence of drug resistance (P=0.79).

Table 3 | Association of the mean level of vitamin E with types of epilepsy and treatment parameters.

Parameters	No.	Vitamin E level (Ug/dl)		T test‡ and P value
		Mean	±SD	
Epilepsy type				
Focal	35	4.32	0.74	t= 0.264
Generalized	15	4.26	0.94	P= 0.793(NS)†
Medication				
Enzyme inducer*	30	4.27	0.71	t= 0.302
Non-enzyme inducer	18	4.35	0.97	P= 0.764(NS)
Drug resistance				
No	33	4.32	0.82	t= 0.260
Yes	17	4.26	0.76	P= 0.796(NS)
Number of anti-epileptics taken				
No drug	2	4.35	0.77	F= 0.584
One	20	4.29	1	P= 0.629(NS)
Two	18	4.46	0.66	
More than two	10	4.04	0.55	

* Carbamazepine, Oxcarbazepine, Clobazam, phenobarbital, primidon, phenytoin, Lamotrigine, and Topiramate.[20]
‡ : Independent sample t test & ANOVA have been used to test the mean difference
† : NS Not statistically significant

Table 4 | Correlation of the mean level of vitamin E to the mean level of age, duration of epilepsy, and the duration of anti-epileptics used.

Variables	(Mean ± SD)	Vitamin E level (ug/dl) (4.30 ± 0.79)	
		Pearson r	P value
Age/ years	(20.94 ± 10.63)	+ 0.037	0.801(NS)
Epilepsy duration/ years	(11.97 ± 10.05)	+ 0.055	0.704(NS)
Medication duration/ months	(62.13 ± 82.33)	- 0.126	0.392(NS)

- Pearson correlation test used to define the correlations
- NS: Not statistically significant

Table 4 shows the correlation between vitamin E levels in epileptic patients and the age, duration of epilepsy and duration of using anti-epileptic drugs. No significant correlation was found among all these associations with a p-value of 0.801, 0.704, and 0.392 respectively.

DISCUSSION

Deficiency of vitamin E has been reported in patients with epilepsy. It has been attributed to anti-epileptic therapy, and however the anti-epileptic effect of Vitamin E is contrary.[21] This study aimed to assess the association of vitamin E levels of patients with epilepsy compared to persons without. Also, we measured the association of the level of vitamin E with the type of epilepsy, its duration, the type and duration of medications used and drug resistance.

Our findings showed that the mean level of vitamin E in patients with epilepsy was (4.3±0.79) µg/dl while the mean in persons without was (11.03±4.08) µg/dl, this finding was statistically significant (P=0.0001). Higashi et al16 found a similar result in their study.[16]

In our study, we found that the mean level of vitamin E is higher in patients with focal epilepsy than in those with generalized one 4.32±0.74 versus 4.26±0.94 µg/dl: however, this difference was statistically non-significant with a p-value of 0.793. Lower mean levels of

vitamin E in patients with generalized epilepsy could be due to the increase in oxidative stress that occurs during epilepsy.[22,23]

The medications used by our patients were divided into two main groups: Enzyme inducers like Carbamazepine, Clobazam, Phenytoin, Topiramate>200 mg, and Lamotrigine>200 mg) and Non-enzyme inducers like Valproate, levetiracetam, Clonazepam, Lacosamide, Topiramate<200 mg, Lamotrigine<200 mg.[24] Although the mean level of vitamin E in patients using Enzyme inducers is lower than in those using Enzyme inhibitor , no statistically significant association was found, with a p-value of 0.76. This result could be due to the presence of more than one mechanism that anti-epileptics could affect the level of vitamin E other than enzyme induction, like the effect on mitochondrial oxidation of fatty acids and impair ATP synthesis, or the effect on carnitine.[25]

Regarding the intake of anti-epileptic medications, we divided our patients into four main groups: no medication (newly diagnosed cases only), one medication, two medications and more than two medications; No statistically significant association was found between the mean level of vitamin E and the four groups, with a p-value of P=0.629. The small size of each group and the possible effect of the disease rather than the medications might explain this result which was contradict that concluded by and this could be Higashi et al.[16]

Two-thirds of our patients were drug-responded epilepsy and one-third were drug-resistant. The mean level of vitamin E was lower in the patients with drug-resistant epilepsy; nevertheless, The study found no significant differences between the two groups, with a p-value of P=0.79.

In this research, we also studied the correlation between the level of vitamin E with the patient's age, duration of epilepsy, and the duration of using anti-epileptics; the differences showed no statistical significance (Table 4). All these findings could point to the correlation between the low level of vitamin E and epilepsy per say rather than the epilepsy type, duration or medications and this correlation could be due to the effect of oxidative stress and the formation of free radicals that were increased during epilepsy which can act as a pathogen in the disease.[26,27]

CONCLUSION

In conclusion, our result suggest that vitamin E deficiency occur in epilepsy as the effect of the disease itself rather than the effect of medications, type of epilepsy, or duration of the epilepsy because of the power of the oxidative stress that had been found to increase during epilepsy. We recommend starting new researches to study the effect of vitamin E as antiepileptic medication.

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Abbreviations list: Antiepileptic drugs (**AEDs**), Deoxyribonucleic acid (**DNA**), High-performance liquid chromatography (**HPLC**), Standard Deviation (**SD**), Valproate (**VPA**).

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