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RESEARCH

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Assessment of serum complement level in a sample of patients of idiopathic childhood epilepsy

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Abstract

Background The etiology of epilepsy is still unknown in over a third of cases and a third of patients have seizures resistant to current antiseizure drugs. Most antiseizure drugs work on suppressing seizures, not targeting the underlying pathophysiological mechanisms because these mechanisms are incompletely understood. Understanding the process of epileptogenesis may lead to pathophysiology-driven drug development of more effective treatment. The aim of this study is to assess the role of the immune system in children with epilepsy, using complement as an immune marker.

Results The serum complement level in the cases group ranged from 1.8 to 4.5 mg/ml, with mean value 2.850 ± 0.646 mg/ml. While in the control group ranged from 2.7 to 26 mg/ml, with mean value 9.208 ± 4.805 mg/ml. The study showed a statistically significant decrease in C3 serum level in cases compared to control group with P-value < 0.001. Also, there was no statistically significant relation between seizure control and serum C3 level.

Conclusion To conclude, it was found that complement component C3 levels are significantly lower in idiopathic childhood epilepsy patients in relation to control group.

Keywords Epilepsy, Complement, Child, Immune system, Antiseizure drug

Background

Epilepsy is a serious neurologic condition associated with stigma, psychiatric comorbidity, and high economic burden [1]. The WHO's 2010 Global Burden of Disease study ranks epilepsy as the second most burdensome neurologic disorder worldwide in terms of disability-adjusted life years [2]. Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition [3]. The etiology of epilepsy is still unknown in over a third of cases and a third of patients have seizures resistant to current antiseizure drugs [4]. Most antiseizure drugs work on suppressing seizures, not targeting the underlying pathophysiological mechanisms because these mechanisms are incompletely understood [5, 6]. Understanding the process of epileptogenesis may lead to pathophysiology-driven drug development of, possibly, more effective treatment [7]. Converging experimental and human studies suggest that the immune system has a critical role in epileptogenesis [8, 9]. Consequently, the involvement of different immune pathways in epilepsy pathogenesis is increasingly investigated in both animal models and in humans [10]. The complement system is an important component of the human immune response and an essential effector of both humoral and cellular

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immunity [11]. The effector of the complement system is the complement factor C3, which changes its serum concentrations in response to the activation of the complement cascade [12]. Dysregulation of the complement system in epilepsy has been observed both in human and animal studies [4]. A clearer understanding of underlying pathogenic mechanisms causing epilepsy would enable more effective treatment strategies, targeted towards these mechanisms [13].

Methods

This is a case control study, including 50 patients attending the pediatric neurology clinic and 50 age and sex matched controls. Inclusion criteria: age range: 2–18 years old, gender: both sexes, nationality: Egyptian, diagnosed epilepsy according to—International League Against Epilepsy 2017 classification of seizure types [14]. All patients have no abnormality in brain MRI. Treated and untreated children were included. Exclusion criteria: chronic liver disease, use of corticosteroids over the 4 weeks preceding blood sample, autoimmune disorders, recent infections, seizures in the 24 h before the blood sample, to exclude complement factors changes owing to ictal conditions. Sample size was calculated by consulting a statistician and found that a sample size of at least 34 cases per group achieves a power of 80% to detect an effect size of 0.7 using two-independent samples t-test with level of significance of 0.05. Ethical consideration: a consent was asked from each patient or their guardians participating in the study (the study was explained to the patient or his legal guardian). Study procedures: all patients were subjected to history taking including: age, gender, past medical history, age of onset of disease, type of convulsion (focal, generalized), frequency of seizures, current medical treatment. Long-term EEG for 2 h was done using the system Nicolet EEG v5.71.6.2577, 2011, VIASYS Healthcare Inc., USA, video recording was not done. Brain MRI was done using Philips MR system Inginea 1.5 T. All subjects participating in this study were subjected to laboratory analysis of serum complement component C3, three milliliters of venous blood were withdrawn from each patient. The collected samples were analyzed using the Human Complement 3 ELISA kits, manufactured by Bioassay Technology Laboratory, Shanghai. This ELISA kit applies to the in vitro quantitative determination of human Complement 3 concentration in serum. This kit has: sensitivity of 0.025 mg/ml, detection range (0.05–30 mg/ml). All data was recorded, entered, and analyzed using the statistical package of social sciences (SPSS) version 23.

Results

This study is a cross-sectional observational study conducted between March 2022 and August 2022, aiming to assess serum complement component C3 in patients with idiopathic childhood epilepsy in comparison to age and sex matched controls and analyze its correlation with age of onset, seizure frequency, and antiseizure drugs used. The total sample was divided into group with epilepsy and healthy group. The ages of the cases group were between 2 and 16 years and those of the control group were between 2 and 17. The mean age of the group with epilepsy was 9.747, while that of the control group was 8.680 showing no statistically significant difference. There was a slightly higher prevalence of male subjects (56% of the total sample) in both groups (Table 1). The study included 50 children with epilepsy, 16 of them were fit free for the past month, while 34 had fits in the past month. The number of fits experienced by these 34 patients ranged between 1 and 300 (once per month to 10 times per day). The age of onset of seizures of the studied sample ranged from 1 and 186 months (15.5 years).

Table 1 Demographic data of the sample

	Group		T-test			
	Cases	Control	t	P-value		
Age (years)						
Range	2–16	2–17	1.428	0.157		
Mean ± SD	9.747 ± 3.586	8.680 ± 3.879				
Chi-square	**N**	**%**	**N**	**%**	**t**	**P-value**
Sex						
Male	28	56.00	28	56.00	0.000	1.000
Female	22	44.00	22	44.00		

SD standard deviation

Statistical tests: (mean and standard deviation, Chi-square, T-test)

p-value (0.157 and 1.00): nonsignificant

Table 2 Number of antiseizure medications received by cases group

Treatment	N	%
No treatment	2	4.00
Monotherapy	31	62.00
Polytherapy	17	34.00
Total	50	100.00

N number

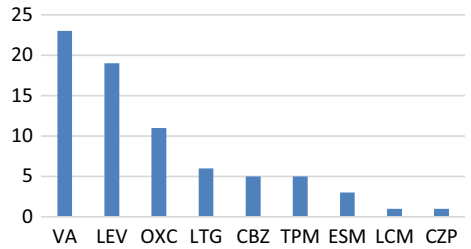

Fig. 1 Antiseizure drugs received by patients. *CBZ* carbamazepine, *CZP* clonazepam, *ESM* ethosuximide, *LCM* lacosamide, *LMT* lamotrigine, *OXC* oxcarbazepine, *TPM* topiramate, *VA* valproic acid

Table 3 Types of antiseizure medications received by cases group

Treatment	N	%
VA	23	46.00
LTG	6	12.00
ESM	3	6.00
LEV	19	38.00
CZP	1	2.00
TPM	5	10.00
CBZ	5	10.00
LCM	1	2.00
OXC	11	22.00

CBZ carbamazepine, *CZP* clonazepam, *ESM* ethosuximide, *LCM* lacosamide, *LMT* lamotrigine, *N* number, *OXC* oxcarbazepine, *TPM* topiramate, *VA* valproic acid

The study included 31 patients with focal epilepsy and 19 patients with generalized epilepsy according to clinical and EEG finding. Of the 50 patients included in the study, 2 patients were on no treatment, 31 patients were on one antiseizure drug and 17 patients were on more than one antiseizure drug (Table 2). Regarding treatment, 23 patients were receiving Valproic acid (VA) either alone or in combination with other drugs, 19 patients were on Levetiracetam (LEV), 11 patients were on Oxcarbazepine (OXC), 6 patients were on Lamotrigine (LTG), 5 were on Carbamazepine (CBZ), 5 were on Topiramate (TPM), 3 were on Ethosuximide(ESM),1 on Lacosamide (LCM), and 1 on Clonazepam(CZP) (Fig. 1; Table 3). The serum

Table 4 C3 level in the studied sample

C3 (mg/ml)	Group		T-test	
	Cases	Control	t	P-value
Range	1.8–4.5	2.7–26	−9.274	< 0.001*
Mean ± SD	2.850 ± 0.646	9.208 ± 4.805		

Statistical tests: (mean and standard deviation, Chi-square, T-test)

p-value (< 0.001): significant

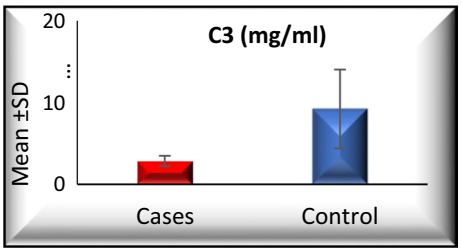

Fig. 2 Comparison between the two groups regarding C3 level

complement level in the cases group ranged from 1.8 to 4.5 mg/ml, with a mean value 2.850 ± 0.646 mg/ml. While in the control group ranged from 2.7 to 26 mg/ml, with mean value 9.208 ± 4.805 mg/ml (Table 4). The study showed a statistically significant decrease in C3 serum level in cases in relation to control group with P-value < 0.001 (Fig. 2). The study showed no significant difference in C3 level between males and females, or between focal and generalized epilepsies. Moreover, there was no significant difference in serum C3 level between patients on no treatment, on one antiseizure drug, and more than one antiseizure drug. Also, the study showed no significant difference in serum C3 level between patients receiving Valproic acid, either alone or in combination, and those who are not. In the studied sample, neither age nor the age of onset of seizures showed a statistically significant relation with serum C3 level. Also, there was no statistically significant relation between number of fits per month and serum C3 level.

Discussion

Complement is an essential component of the innate immune system. However, dysregulation or inadvertent activation of complement components contribute to the pathogenesis of some neurological disorders [11]. In this study, we aimed to assess the serum complement component C3 level in children with epilepsy with different epilepsy types, on different antiseizure medications in a sample of Egyptian children and adolescent attending pediatric neurology outpatient clinics. The effector of the complement system is the complement factor C3,

which changes its serum concentrations in response to the activation of the complement cascade [12]. Accordingly, complement system activation produces the reduction of C3 serum levels, which physiologically acts on the following factors of the complement cascade [8]. The current study found significantly reduced serum C3 levels in children with epilepsy in comparison to age and sex matched controls. This finding is similar to the finding of the study conducted by Liguori et al. [8] which included 37 idiopathic generalized epilepsy patients and showed significant reduction of C3 and C4 serum concentrations in idiopathic generalized epilepsy patients. Furthermore, similar to Liguori et al. [8], we did not document significant differences in serum C3 levels between seizure free epileptic patients and patients with persistent seizures, and also, there was no significant correlation between seizure frequency and C3 serum level. Although our analysis is limited by the heterogeneous sample of patients with persistent seizures due to the different seizure frequency in each subject, this finding needs to be further analyzed in larger populations of patients. Moreover, the current study found no significant difference in C3 serum level between patients with focal and generalized epilepsies; this may partly be accounted for by the small number in each subgroup. Complement system dysregulation has been documented in both focal epilepsy, especially temporal lobe epilepsy [15, 16], and generalized epilepsy [8]. In contrast to our findings, Liguori et al. [8] found significantly lower C3 and C4 serum levels in untreated Idiopathic generalized epilepsy patients compared to treated patients, this may be explained by the fact that our study included only two untreated children with epilepsy out of 50 cases while Liguori et al. included 9 untreated cases out of 37. On the other hand, Hincal et al. [17] conducted a study including 90 idiopathic epilepsy patients (38 were on no treatment, 30 were on Phenytoin monotherapy, and 22 on Carbamazepine monotherapy) and found that the mean levels of C3 complement proteins were significantly higher than healthy controls in untreated people with epilepsy and Carbamazepine treated patients, these contradictory results may possibly be due to the different laboratory analysis technique used by Hincal et al., the radial immunodiffusion technique on Nor-Partigen plates, this difference may also be because Hincal et al. sampled after a minimum seizure free period of 5 days. It appears that the inconsistent and controversial data existing in this field may be the result of the study designs. A better design would involve age and sex matched healthy controls and include measurement of complement levels both before and during every single antiseizure drug therapy at determined intervals, so that abnormalities possibly related to epilepsy itself could be differentiated from those induced by an antiseizure drug. Furthermore, alterations of the immune system have been reported in patients receiving some antiseizure drugs. The reported changes suggest a reduced response of the immune system and inflammatory components (transient immunodeficiency) in patients receiving carbamazepine [18] and reduced production of Tumor Necrosis Factor-alpha and Interleukin-6 by sodium valproate [19]. Moreover, numerous genetic, environmental, and lifestyle factors such as discrepancies in hormones, obesity, and exposure to smoking may influence the serum levels of complement components especially since a single determination is performed [20]. In fact, our findings do not allow determining the direction of causation between complement system dysregulation and epilepsy, as inflammation promotes blood brain barrier compromise and promotes seizures and seizures in turn add further to the blood brain barrier damage and feed the inflammatory response [21, 22], thus needing further investigations aimed at evaluating the role of complement cascade in epilepsy pathogenesis and clinical consequences. Studying brain inflammation could be exploited for therapeutic purposes, for example, to identify the patient population with more significant brain inflammation since these patients might benefit from specific targeted therapies adjunctive to antiseizure drugs [23]. Moreover, brain inflammation could be used as a biochemical marker of the therapeutic success of a treatment with disease-modifying properties [24]. The limitations of the current study include (1) the small sample size considering the incidence of childhood epilepsy [2]. (2) The cross-sectional way to study patients, a better way would be to assess serum complement level before and after achieving seizure control and before and after receiving antiseizure drug medication [4]. However the limited duration of this study was the reason. (3) The small number in each subgroup (patients with generalized or focal epilepsy, patients receiving each antiseizure drug) did not enable us to detect differences between them.

Conclusion

To conclude, it was found that complement component C3 levels are significantly lower in idiopathic childhood epilepsy patients in relation to control. Also, the study showed that there is no significant difference in C3 level in serum between studied population on different antiseizure drugs, and between epileptic patients with controlled seizures and those with uncontrolled seizures.

Abbreviations

C3	Complement component 3
CBZ	Carbamazepine
CZP	Clonazepam
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
ESM	Ethosuximide

LCM	Lacosamide
LMT	Lamotrigine
MRI	Magnetic resonance imaging
OXC	Oxcarbazepine
SPSS	Statistical package of social sciences
TPM	Topiramate
VA	Valproic acid
WHO	World Health Organization

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Author contributions
FA: data collection and research project execution. MM: contribution to the concept and design, drafting the manuscript. AN: conception of the work, manuscript revision. NS: conception of the work, Approved the version to be published. All authors have agreed to conditions noted on the Authorship Agreement Form and have read and approved the final version submitted. The content of the manuscript has not been published, or submitted for publication elsewhere.

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Availability of data and materials
All raw data will be available on the editor request through communication with the corresponding author.

Declarations

Ethics approval and consent to participate
All procedures performed in the study were in accordance with the ethical standards of the faculty of medicine, Ain Shams university research and ethical committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval was obtained from faculty of medicine, Ain Shams university research and ethical committee FWA 000017585, headed by Professor Fathy Tash, on the 8th of March, 2022. Written informed consent was obtained from participants or their guardians for participation.

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no competing interests.

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