European Journal of Inflammation

Letter to the Editor

European Journal of Inflammation
Volume 16: 1–6

DOI: 10.1177/2058739218808165
journals.sagepub.com/home/eji
SAGE

Serum level of C-reactive protein and interleukin-6 in children with drug-resistant epilepsy

Mohamed M Elwan,[1] Nirmeen A Kishk,[2] Rasha A El-Kapany,[1] Ibrahim E Al-Ahmer[1] and Ahmed Elkady[3]

Abstract

There is increasing evidence that chronic inflammation affects the pathophysiology of epilepsy, especially the drug-resistant type. Drug-resistant epilepsy is a challenging condition, because of the difficulties in its management, and its unclear epileptogenesis. This study is looking at C-reactive protein (CRP) and interleukin-6 (IL-6) levels in those with drug-resistant epilepsy and the correlation of these levels with seizure frequency. Hence, 40 children with drug-resistant epilepsy were included in this study and compared with 20 healthy volunteers (as a control group). Participants were aged between 5 and 15 years. Patients were divided into two subgroups, those with daily seizures (Group A1) and those with monthly seizures (Group A2). Serum levels of CRP and IL-6 were measured in all participants. The clinical characteristics, electroencephalography, and magnetic resonance imaging (MRI) findings were then compared. CRP levels were significantly higher in Group A1, at 21.88–93.29 mg/L than both Group A2 and the control group, at 3.02–40.37 mg/L and 2.23–13.18 mg/L, $P < 0.01$ and $P < 0.001$, respectively. The IL-6 levels were also significantly higher in Group A1, at 153.60–597.80 ng/L than in both Group A2 and the control group, at 97.40–232.50 ng/L and 12.00–96.30 ng/L, $P < 0.01$ and $P < 0.001$, respectively. Significantly higher levels of CRP and IL-6 were associated with earlier age of onset ($P < 0.01$), seizure frequency ($P < 0.05$), and the frequency of status epilepticus ($P < 0.01$). Moreover, frequent-generalized motor seizures are correlated with elevated CRP and IL-6 levels. As a result, this systemic inflammatory reaction in children may contribute to drug-resistant seizure and potentially could be used as biomarkers to be correlated with disease severity and prognosis.

Keywords

C-reactive protein, drug-resistance, epilepsy, inflammation, interleukin-6

Date received: 21 April 2018; accepted: 13 September 2018

Introduction

Epilepsy is one of the most common pediatric neurological disorder, with 4%–10% of children suffering from one seizure at least in the first 16 years of their lives.[1] Drug-resistant epilepsy at an early stage of the disease may be harmful to the brain development due to its interference with developmental milestones.[2] Epilepsy's pathophysiology has remained unclear in several regards, leading to insufficient seizure control in almost one-third of patients, as most antiepileptic drugs (AEDs) are directed to control seizures and not to cure epilepsy itself.[3] There is accumulating data from human studies and experimental models suggesting that

[1]Neurology Department, Faculty of Medicine, Menoufia University, Shibin El Kom, Egypt
[2]Neurology Department, Faculty of Medicine, Cairo University, Cairo, Egypt
[3]Neurology Department, Mataria Teaching Hospital, General Organization of Teaching Hospitals & Institutes (GOTHI), Cairo, Egypt

Corresponding author:
Ahmed Elkady, Neurology Department, Mataria Teaching Hospital, General Organization of Teaching Hospitals & Institutes (GOTHI), 2nd Floor, 7 Taha Kandil Street, Mataria, Cairo 11719, Egypt.
Email: ahkady86@gmail.com

the pathophysiology of epilepsy involves inflammatory process, and these studies show that frequent seizures could induce the production of inflammatory mediators, which in turn influence both the course and the pathogenesis of epilepsy.[4] C-reactive protein (CRP) is an acute-phase short pentraxin protein that is sensitized in the liver after exposure to inflammatory signals. Initially, it was recognized as an inflammatory marker. However, there are not many studies of CRP in the context of seizures or epilepsy.[5] Interleukin-6 (IL-6), however, is a pleiotropic multifunctional cytokine, which has many biologic actions on various cell types and tissues. Many clinical studies demonstrated that levels of IL-6 could increase after not only focal seizures but also after secondarily generalized epileptic seizures.[6] Thus, this study investigates serum levels of CRP and IL-6 in patients with drug-resistant epilepsy compared to the other groups and the relation of these levels with seizure frequency.

Materials and methods

Patients

This study was conducted on 40 Egyptian children with epilepsy and 20 healthy controls. Patients' clinical data were evaluated and those who met the following inclusion criteria were included: (1) children with clinical diagnosis of epilepsy based on electro-clinical findings (seizure semiology and electroencephalogram (EEG)), who have primary generalized motor seizures or focal seizures with secondary generalization, according to the criteria of International League against Epilepsy (ILAE) 2010 guidelines. (2) Children with drug-resistant epilepsy, according to the definition of drug-resistant epilepsy of ILAE in 2010.[7] During the period of this study between February 2016 and November 2016, 257 children with epilepsy aged 5–15 years were interviewed; of those, 217 were excluded due to the following: (1) they were controlled under treatment, without any seizures. (2) Their imaging showed focal structural lesions as ischemic, hemorrhagic encephalomalacia, vascular malformation, traumatic lesions, or any congenital anomalies. (3) They had focal neurological deficit on examination. (4) Patients with acute infectious illness or inflammation at the time of the interview. Finally, we included 40 patients with drug-resistant epilepsy, mainly of generalized motor type (Figure 1). We classified our patients who were included

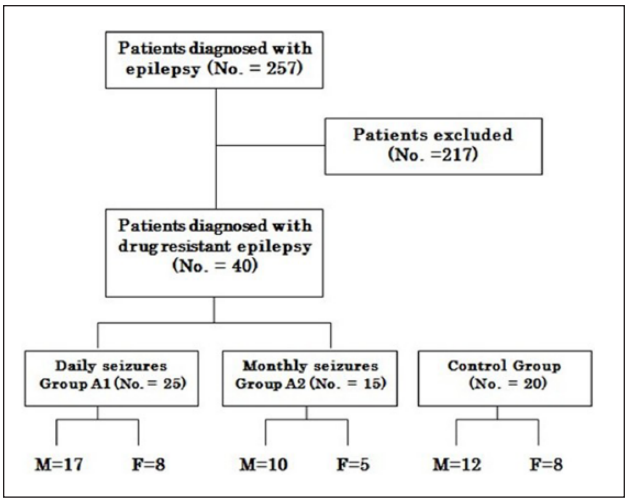

Figure 1. Flow diagram of participants in this study.

(40 patients) into two subgroups according to seizure frequency, a daily seizures group (Group A1), and a monthly seizures group (Group A2). Group A1: included 25 patients (17 males and 8 females). Group A2: included 15 patients (10 males and 5 females). Patients were recruited from Cairo University Epilepsy Clinic (CUEC) Kasr al-Aini Hospital and epilepsy Outpatient Clinic of Menoufia University Hospital. Group B was made up of 20 (12 males and 8 females) normal children age and sex matched healthy volunteers. All patients underwent: (1) a full neurological examination to exclude any focal neurological deficit, (2) an awake interictal long-term EEG under normal standard condition according to 10–20 international system of electrode placement, using mono and bipolar montages. (3) A brain magnetic resonance imaging (MRI) to exclude any central inflammation or infection. (4) Full routine blood tests, including complete blood count, liver function tests, kidney function tests, and serum electrolyte levels in an attempt to exclude any other systemic infectious or inflammatory diseases. No patient in either subgroup had undergone any immune-related treatments, such as adrenocorticotropic hormone (ACTH), glucocorticoid, or immunoglobulin therapies, in the last 6 months. Written informed consent from all patient guardians had been obtained. The ethical committee of Menoufia University hospital approved the study design.

Determination of serum CRP and IL-6 levels

Fasting samples were collected for IL-6 and CRP level measurement (time of the blood sample from

last seizure >6 h and >48 h from status epilepticus, as they are sampled from stable patients at their follow up in the outpatient clinic). Blood samples (3 mL) were withdrawn from peripheral veins, left at room temperature for 30 min then centrifuged at 3000 r/min for 10 min to separate the sera, to analyze levels of CRP and IL-6 by enzyme-linked immunosorbent assay (ELISA). Sera were stored at −20°C till the time of assay. CRP and IL-6 concentrations were determined using commercially available kits (Immunospec Corporation (sunred) Co., China). The principal of the assay for CRP and IL-6 was ELISA for the detection and quantification of biological molecules secreted or released by cells. This method immobilizes and binds a target-specific captured antibody onto a high protein by ELISA plate, which in turn enables the capture of target protein. The captured protein is then detected by a protein-specific biotinylated antibody using Stat Fax 2100 microplate reader.

Statistical analyses

The data were summarized using mean and standard deviation (SD) for quantitative data with parametric distribution, median with interquartile range (IQR) for quantitative data with non-parametric distribution and number with percentages for qualitative data. When comparing two groups with quantitative data and parametric distribution, independent t-test is used. While comparing between more than two groups, one-way analysis of variance (one-way ANOVA) was used. Mann–Whitney U test was used to compare quantitative data with non-parametric distribution between two groups, while Kruskal–Wallis test was used between more than two groups. The chi-square test was used to compare groups according qualitative data. Logistic regression analysis was used to assess the independent risk factors of higher inflammatory markers. The confidence interval (CI) was set to 95% and the margin of error accepted was set to 5%, and a $P < 0.05$ was considered statistically significant.

Results

Clinical results

All participants were age and sex matched. The main clinical, electrophysiological, and MRI differences between the two patient subgroups, daily seizure group (n = 25) and monthly seizure group (n = 15), are summarized in Table 1.

Patients with daily seizures had a younger age of onset of seizures (mean = 3.42 ± 1.80) than those with monthly seizures (mean = 5.80 ± 1.74), $P = 0.004$. The duration between seizure attacks (frequency of seizures) was shorter in daily seizures group (median (IQR) = 1 day) than in monthly seizures group (median (IQR) = 21 days) with $P < 0.001$. Number of status epilepticus or serial seizures, which required admission to hospital per year, was more in daily seizures group (mean = 16.27 ± 3.54) than in monthly seizure group (mean = 2.00 ± 0.00) with $P < 0.001$. All patients in daily seizures group were on polytherapy of 3–4 antiepileptics compared to 2–3 in patients of monthly seizures group, with $P = 0.007$. Myoclonic seizures were common in patients with daily seizures (10 patients), whereas no patient with monthly seizures had myoclonic seizures with $P = 0.005$. There was a difference between EEG findings in both groups with $P = 0.009$.

Inflammatory markers results

All initial routine laboratory results were within normal ranges, and inflammatory markers investigations show that levels of CRP were significantly higher in patients' groups (median (IQR) = 27.43 mg/L) than in control group (median (IQR) = 4.48 mg/L) with $P < 0.001$. Similarly, IL-6 levels were significantly higher in patients' groups (median (IQR) = 247.30 ng/L) than in control group (median (IQR) = 46.60 ng/L) with $P < 0.001$.

Among patients subgroups and control group, levels of CRP were significantly higher in daily seizure group (median (IQR) = 33.37 mg/L) than both monthly seizure group (median (IQR) = 20.92 mg/L) and control group (median (IQR) = 4.48 mg/L) with $P = 0.007$ and $P < 0.001$, respectively; also, CRP levels were significantly higher in monthly seizure group than control group with $P = 0.002$ (Figure 2).

IL-6 levels were significantly higher in daily seizure group (median (IQR) = 299.80 ng/L) than both monthly seizure group (median (IQR) = 142.20 ng/L) and control group (median (IQR) = 46.60 ng/L) with $P = 0.005$ and $P < 0.001$, respectively; also, IL-6 levels were significantly higher in monthly seizure group than control group with $P = 0.003$ (Figure 3).

Table 1. Clinical, electrophysiological, and MRI characteristics of patients groups.

		Monthly seizures group (n = 15)	Daily seizures group (n = 25)	*P* value
Age of onset (year)	Mean ± SD	5.80 ± 1.74	3.42 ± 1.80	0.004*
	Range	3–9	1–8	
Duration of illness (year)	Mean ± SD	4.80 ± 2.68	6.38 ± 2.66	0.077*
	Range	2–9	3–11	
Duration between seizure attacks (day)	Median (IQR)	21 (14–30)	1 (1–1)	0.0003**
	Range	7–90	0–2	
Number of status epilepticus (year)	Mean ± SD	2.00 ± 0.00	16.27 ± 3.54	0.0001*
	Range	2–2	11–24	
Type of seizures	1ry GTCs	11 (73.33%)	20 (80.0%)	0.625***
	Myoclonic	0 (0.0%)	10 (40.0%)	0.005***
	Tonic	2 (13.33%)	8 (32.0%)	0.187***
	Focal with 2ry GTCs	4 (26.66%)	5 (20.0%)	0.625***
Etiology of epilepsy	Idiopathic (genetic)	11 (73.3%)	8 (32.0%)	0.202***
	Symptomatic	0 (0.0%)	4 (16.0%)	
	Unknown	4 (26.7%)	13 (52.0%)	
Number of antiepileptic drugs	Two	12 (80.0%)	0 (0.0%)	0.007***
	Three	3 (20.0%)	19 (76.0%)	
	Four	0 (0.0%)	6 (24.0%)	
EEG finding	Normal	3 (20.0%)	0 (0.0%)	0.009***
	Generalized abnormality	5 (33.33%)	19 (76.0%)	
	Focal abnormality	7 (46.66%)	6 (24.0%)	
MRI finding	Normal	15 (100.0%)	21 (84.0%)	0.102***
	Temporal sclerosis	0 (0.0%)	4 (16.0%)	

MRI: magnetic resonance imaging; SD: standard deviation; IQR: interquartile range; EEG: electroencephalogram.
*Independent t-test; **Mann–Whitney U test; ***Chi-square test.

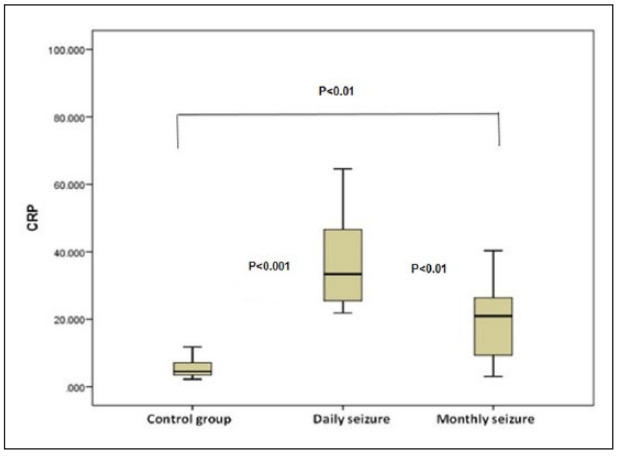

Figure 2. Difference between CRP (mg/L) level in both patients subgroups and control group.

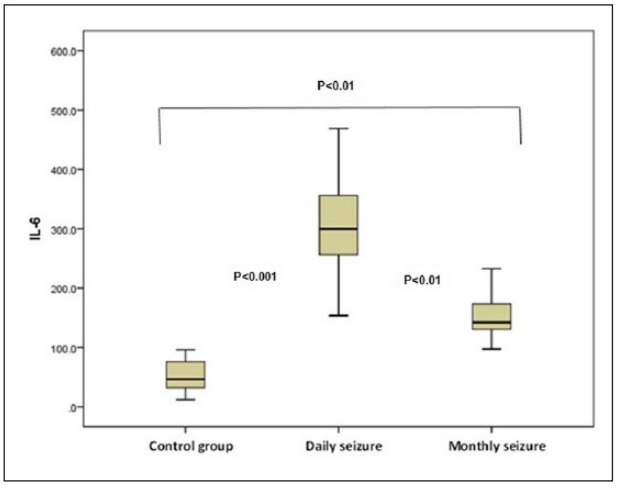

Figure 3. Difference between IL-6 level (ng/L) in both patients subgroups and control group.

Independent risk factors of higher inflammatory markers

Logistic regression analysis was used to analyze different clinical, electrophysiological, and imaging of patient subgroups and their relation to inflammatory mediators investigated; the results showed that age of epilepsy onset (odds ratio (OR): 0.47, 95% CI: 0.29–0.78), duration between attacks (OR: 1.26, 95% CI: 1.16–1.95), and number of status epilepticus per year (OR: 2.12, 95% CI: 1.12–5.17) are the risk factors mostly associated with higher inflammatory markers with significant statistical analysis ($P = 0.003$, 0.021, and 0.004, respectively). Increasing levels of either

CRP (OR: 1.18, 95% CI: 1.04–1.33) or IL-6 (OR: 1.05, 95% CI: 1.01–1.08) is associated with increasing levels of the other with significant statistical analysis ($P=0.007$ and 0.003, respectively). Other factors as number of AEDs used, myoclonic seizures, and EEG changes were not found to be as significant independent risk factors of higher inflammatory markers.

Discussion

There is much research that suggests the crucial role that inflammation plays in both course and pathogenesis of epilepsy; however, the exact mechanism is still unknown.[8] The increase in IL-6 serum levels which in turn induce CRP production from hepatocytes indicating chronic inflammation. In children with drug-resistant epilepsy, this is possibly due to the effect of daily multiple generalized motor seizures on sustaining the production of IL-6 before its degradation.[6] Increased CRP and IL-6 concentrations in patients with generalized motor seizures without any evidence of systemic or central nervous system infections or inflammation, compared with healthy controls, shows higher prevalence in patients with daily seizures compared to those with monthly seizures. This suggests that seizures could be a progressive disorder, leading to neuropathologic changes that in turn increase the severity of epilepsy.

Our results were similar to the results of Ishikawa et al. in 2015, who found that levels of CRP and IL-6 were higher in daily seizure patients compared to monthly seizure patients and healthy controls. However, it is worth noticing that a significant difference was found between CRP and IL-6 levels in monthly seizure patients and healthy controls in this study, whereas in Ishikawa et al.,[9] such difference was insignificant, and this may be due to the variation of sampling time in relation to seizures in this group.

In this study, it was found that frequency of seizures per day and number of status epilepticus per year are independent risk factors for increasing inflammatory mediators, besides early age of onset. Many studies agree with such results, correlating high seizure frequency with elevated IL-6 levels and consider drug-resistant epilepsy with daily seizures to have higher significant levels of IL-6 than less frequent seizures.[9,10]

No association was found in this study between AEDs polytherapy and increasing inflammatory marker levels. One study revealed no significant relationship between the number of AEDs used and CRP level.[11] However, another found a significant relation between increase inflammatory markers and AEDs usage explained by longer duration of AED therapy, which leads to rising risk of the acceleration of atherosclerosis in patients with drug-resistant epilepsy on polytherapy, independent from age, gender, and oxidative stress effect on atherosclerotic process.[12] In conclusion, systemic inflammatory response has been detected in children with drug-resistant epilepsy. This systemic inflammation may affect course as well as prognosis of their illness, so measuring and follow-up of these biomarker levels may be of value in those intractable patients.

Acknowledgements

The authors thank all parents and patients as well as colleagues involved in the medical care of our patients.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Friedman MJ and Sharieff GQ (2006) Seizures in children. *Pediatric Clinics of North America* 53: 257–277.
2. Chen YH, Kuo TT, Chu MT et al. (2013) Postnatal systemic inflammation exacerbates impairment of hippocampal synaptic plasticity in an animal seizure model. *Neuroimmunomodulation* 20: 223–232.
3. Li G, Bauer S, Nowak M et al. (2011) Cytokines and epilepsy. *Seizure* 20: 249–256.
4. Vezzani A, Friedman A and Dingledine RJ (2013) The role of inflammation in epileptogenesis. *Neuropharmacology* 69: 16–24.
5. Alapirtti T, Waris M, Fallah M et al. (2012) C-reactive protein and seizures in focal epilepsy: A video-electroencephalographic study. *Epilepsia* 53: 790–796.
6. Uludag IF, Bilgin S, Zorlu Y et al. (2013) Interleukin-6, interleukin-1 beta and interleukin-1 receptor antagonist levels in epileptic seizures. *Seizure* 22: 457–461.

7. Kwan P, Arzimanoglou A, Berg AT et al. (2010) Definition of drug resistant epilepsy: Consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 51: 1069–1077.
8. Vezzani A, French J, Bartfai T et al. (2011) The role of inflammation in epilepsy. *Nature Reviews Neurology* 7: 31–40.
9. Ishikawa N, Kobayashi Y, Fujii Y et al. (2015) Increased interleukin-6 and high-sensitivity C-reactive protein levels in pediatric epilepsy patients with frequent, refractory generalized motor seizures. *Seizure* 25: 136–140.
10. Lehtimäkia KA, Liimatainenb S, Peltola J et al. (2011) The serum level of interleukin-6 in patients with intellectual disability and refractory epilepsy. *Epilepsy Research* 95: 184–187.
11. Yuen AW, Bell GS, Peacock JL et al. (2010) Effects of AEDs on biomarkers in people with epilepsy: CRP, HbA1c and eGFR. *Epilepsy Research* 91: 187–192.
12. Tan TY, Lu CH, Chuang HY et al. (2009) Long-term antiepileptic drug therapy contributes to the acceleration of atherosclerosis. *Epilepsia* 50: 1579–1586.