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Evaluation of Acetylcholinesterase and Acetylcholine Levels in Children with Idiopathic Epilepsy

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Objective of this work was to assess whether acetylcholinesterase and acetylcholine, levels that can be used as biomarkers for drug-resistant epilepsy in children with idiopathic epilepsy. Acetylcholinesterase and acetylcholine levels were measured in three groups of children, 30 children with drug resistant epilepsy,30 with seizures free and30 age and sex matched healthy children. Significant lower acetylcholinesterase was found in drug resistant epilepsy compared to seizure free epilepsy and healthy controls. Higher acetylcholine levels was found in seizure free epilepsy compared to drug resistant epilepsy and healthy controls. Stepwise linear regression analysis showed that low ACHE, high ACH, high severity score are significant independent factors associated with idiopathic epilepsy. Moreover, Receiver Operating Characteristic (ROC) analysis showed that severity score at cutoff of Chalfont score>60 had the highest sensitivity 86.7% and specificity 80% followed by serum ACHE at cutoff <3.212(ng/ml) with sensitivity 70% and specificity 100% and then serum ACH at cutoff >18.410(ng/ml)with sensitivity 70% and specificity 83.3% as predictors for idiopathic epilepsy. Increased circulating level of ACHand decreased ACHE may predict idiopathic epilepsy suggesting their role in the childhood idiopathic epilepsy'spathogenesis.

Keywords: Acetyl cholinesterase; acetylcholine; idiopathic epilepsy.

Epilepsy affected the development of learning and cognitive functions via a number of numerous factors: etiology [1] age of onset, seizure type[2,3] duration and severity, interictal epileptic form discharges,[4]drug treatment. Epilepsy is a devastating neurological and systemic disorder characterized by recurrent seizures[5]. Despite the rapid progression in clinical and pre-clinical epilepsy research, the pathogenesis of epilepsy still remains elusive Acetylcholinesterase (AChE) has a significant role in the pathogenesis of neurodegenerative diseases by inducing aggregation of pathological proteins, oxidative stress, apoptosis, and inflammatory response. Diminished AChE concentrations cause irregularly augmented concentrations of ACH in cholinergic synapses, producing unnecessary nicotinic receptors and muscarinic stimulation [6]. Researches associated

to ACh on the vagus nerve for epilepsy were imperative to elucidate inflammations in epilepsy. The present work will explore the predictive role of AChEand ACH for idiopathic epilepsy and how it is related to clinical features of the patients.

SUBJECTS AND METHODS

Subjects

The study comprised three groups of children, 30 children with drug resistant epilepsy, 30 with seizures free and 30 age and sex matched healthy children.

Methods

Assessment of Acetyl cholinesterase Activity (AChE)

Acetyl cholinesterase Activity was assessed by a double-antibody sandwich enzyme-linked immunosorbent assay ELISA kit from Shanghai Biovision Co., Ltd, Jufengyuan Road, Baoshan District, Shanghai.

Assessment of acetylcholine Activity

The technique was designated for reversed-phase HPLC separation of acetylcholine of their homologues in serum, united with post columnfluorometric quantification and enzymatic derivatization. The separation happens on a polymeric resin derivatized with hydrophobic moiety and the mobile phase comprises Na_2HPO_4. 3-(*p*-hydroxyphenyl) sodium dodecylsulphate; propionate and post column enzyme reactor comprises immobilized choline oxidase, peroxidase and acetyl cholinesterase. Method is well suited for non-attended automatic operation and free of encountered interferences with electrochemical detection.

Ethical Approval

This research was approved by the Ethical Committee of Al-Azhar University (No: 00782) and followed the World Medical Association's Declaration of Helsinki. Furthermore, each participant in the study signed a written consent after a full description of the study.

Statistical analysis

Statistical analysis was performed using SPSS version 21 for windows. Data were expressed as mean ± standard deviation and compared to t-test to compare between two groups. ANOVA and post hoc tests for comparing between more than 2 groups. Data were expressed as percentages and frequencies, and were analyzed with the two-tailed chi square test.

RESULTS

Chalfont severity score was significantly increases in drug resistance epilepsy cases than seizure free and controls (Table 1). Significant lower levels of AChE was found in drug resistant epilepsy compared to seizure free epilepsy and

Table 1. Electroencephalographic findings and clinical data in children with drug resistant epilepsy & seizure freechildren

	Drug resistant Epilepsy (n=30) Mean ± SD	Seizure Free (n=30) Mean ± SD	Independent T test/ chi square test t/x^2	p-value
Age (years)	9.013±1.426	9.277±0.871	-0.863	0.392
Gender (N, %)	20 (66.7%)	22 (73.3%)	0.317	0.573
Male	10 (33.3%)	8 (26.7%)		
Female				
Age of onset of seizures (years)	3.233±1.670	3.783±2.104	1.122	0.267
Duration of disease (years)	5.687±1.523	6.667±3.384	1.446	0.153
Chalfont severity score	91.733±20.120	48.867±16.950	8.925	<0.0001
Type of epilepsy (N, %)			5.56	0.062
Focal	22 (73.3%)	13 (43.3%)		
Generalized	6 (20%)	13 (43.3%)		
Focal with secondary generalization	2 (6.7%)	4 (12.3%)		

*$p < 0.05$

healthy controls ($p < 0.05$). Lower ACH levels was found in seizure free epilepsy compared to drug resistant epilepsy and healthy controls ($p < 0.05$) (Table 2). Stepwise linear regression analysis showed that ACHE, ACH, severity score are significant independent factors associated with idiopathic epilepsy (Table 3). Moreover, Receiver Operating Characteristic (ROC analysis showed that severity score at cutoff of Chlfont score>60 had the highest sensitivity 86.7% and specificity 80% followed by serum ACHE at cutoff < 3.212 (ng/ml) with sensitivity 70% and specificity 100% and then serum ACH at cutoff > 18.410 (ng/ml) with sensitivity 70% and specificity 83.3% as predictors for idiopathic epilepsy.

DISCUSSION

Proinflammatory mediators could amendexcitability of the neurons and affect neurotransmission causing reduction in the seizures threshold and increase neuronal damage[7,8].Cytokines have been involved as inhibitors and mediators of various forms of neurodegeneration[9,10,11]. Pro-inflammatory cytokine in the innate immune response modulates fundamental processes in the brain [10,12].

Table 2. AChE and ACH serum levels in epilepticchildren and healthy children

	Seizure free Children (n=30) Mean ± SD	Children with drug resistant epilepsy(n=30) Mean ± SD	Healthy children(n=30) Mean ± SD	ANOVA test F	p-value
AChE(ng/ml)	3.646±6.601	1.969±1.129	4.550±2.870	71.656	<0.0001**
ACH (ng/ml)	29.705±18.042	12.818±5.253	10.065±7.811	23.755	<0.0001**

	Post hoc analysis		
	Seizure free vs drug resistantepilepsy	Seizure freevshealthy children	drug resistant epilepsy vs healthy children
AChE(ng/ml)	<0.0001*	0.202	<0.01**
ACH (ng/ml)	<0.0001*	0.375	<0.01**

**p < 0.01

Table 3. Liner stepwise regression analysis for prediction of drug resistantepilepsy in idiopathic epileptic children

Coefficients Model		Unstandardized Coefficients		Standardized Coefficients	t	P-value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	3.449	0.115		30.108	<0.0001*	3.22	3.678
	score	-0.013	0.002	-0.761	-8.925	<0.0001*	-0.017	-0.01
2	(Constant)	3.421	0.102		33.625	<0.0001*	3.217	3.625
	score	-0.008	0.002	-0.467	-4.492	<0.0001*	-0.012	-0.005
	ACHE	-0.03	0.007	-0.427	-4.105	<0.0001*	-0.045	-0.015
3	(Constant)	3.393	0.097		35.093	<0.0001*	3.199	3.586
	score	-0.007	0.002	-0.412	-4.112	<0.0001*	-0.011	-0.004
	ACHE	-0.059	0.013	-0.846	-4.723	<0.0001*	-0.084	-0.034
	ACH	0.014	0.005	0.433	2.799	0.007*	0.004	0.024

*p < 0.05

The influence of neuromodulators implicated in the impulses transmission on the pathogenesis is of immense significanceas epileptic seizures happen with the disturbance of the inhibitory-excitatory balance in the brain. Consequently, mutations/disorders of the elements at the ion channel level and receptor might causestimulus transmission abnormality and epileptic discharges. Diminished levels of AChE cause abnormally elevated levels of ACH in cholinergicsynapses, leading to exaggerated stimulation of nicotinic and muscarinic receptors[13–15]. Neuro transmitters mainly ACH has been involved in the epilepsy's pathogenes is as proved via their amendment in pre-clinical model of epileptic seizure[16]. Amongst

Table 4. Predictive values, specificity and Sensitivity for prediction of drug resistant epilepsy in babies with idiopathic epilepsy

variables	AUC	Cutoff point	Sensitivity	Specificity	95% Confidence Interval	
					Lower Bound	Upper Bound
Chalfont score	0.943	>60	86.7%	80%	0.892	0.994
Serum ACHE (ng/ml)	0.995	<3.212	70%	100%	0.984	1.000
Serum ACH (ng/ml)	0.786	>18.410	70%	83.3%	0.659	0.913

AUC: area under curve;

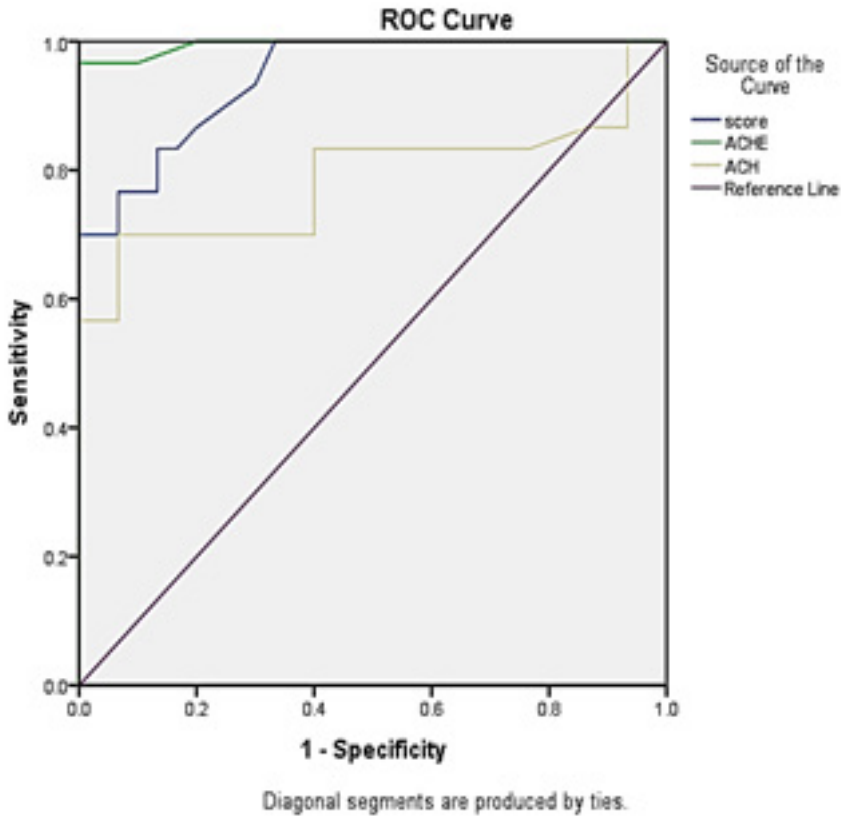

Fig. 1. ROC (Receiver Operating Characteristic) curve for predictors of drug resistant epilepsy in children with idiopathic epilepsy

the various participating influences underlying the seizures generation's mechanism, the role of neurotransmitters has been involved in the same. Neurotransmitters are endogenous constituents that transfer signals across the synapse and controlexcitatory/ inhibitory neuronal functions through fastening to their particular receptors. Usually it is stored in axon terminals, synaptic vesicles and secreted into the synapse following anappropriate signal. Liberated neuro transmitters carry out the connecting functions over the synaptic cleft and fastening to particular receptors. Gut microbiota could modulate brain behavior and function and is highly documented as an imperative factor in mediating the risk of epilepsy and the impacts of seizure interventions[17,18]

CONCLUSION

In conclusion, investigating the relations of ACH and AChE that are the major actors in epilepsy has become an imperative aim to elucidate the underlying pathology in neurological disorders.

Conflicts of Interest

None.

REFERENCES

1. Beghi E, Cornaggia CM, Elia M. Proposal for a multicenter study on epilepsy and learning disorders in children. *Epilepsia*. 2001;42(1):10-12.
2. Bourgeois BFD, Prensky AL, Palkes HS, Talent BK, Busch SG. Intelligence in epilepsy: a prospective study in children. *Ann Neurol Off J Am Neurol Assoc Child Neurol Soc*. 1983;14(4):438-444.
3. Holmes GL. Pathogenesis of learning disabilities in epilepsy. *Epilepsia*. 2001;42:13-15.
4. Binnie CD. Cognitive performance, subtle seizures, and the EEG. *Epilepsia*. 2001;42:16-18.
5. Devinsky O, Vezzani A, O'Brien TJ, et al. Epilepsy Nat Rev Dis Primers 4: 18024. 2018.
6. Aboul Ezz HS, Khadrawy YA, Noor NA. The neuroprotective effect of curcumin and Nigella sativa oil against oxidative stress in the pilocarpine model of epilepsy: a comparison with valproate. *Neurochem Res*. 2011;36(11):2195-2204.
7. Vezzani A, Fujinami RS, White HS, et al. Infections, inflammation and epilepsy. *Acta Neuropathol*. 2016;131(2):211-234.
8. Suleymanova EM. Behavioral comorbidities of epilepsy and neuroinflammation: Evidence from experimental and clinical studies. *Epilepsy Behav*. 2021;117:107869. doi:https://doi.org/10.1016/j.yebeh.2021.107869
9. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci*. 2001;2(10):734-744.
10. Montgomery SL, Bowers WJ. Tumor necrosis factor-alpha and the roles it plays in homeostatic and degenerative processes within the central nervous system. *J neuroimmune Pharmacol*. 2012;7(1):42-59.
11. Rothwell NJ. The role of cytokines in neurodegeneration. In: *Cytokines in the Nervous System*. Springer; 1996:145-162.
12. Zalpoor H, Akbari A, Samei A, et al. The roles of Eph receptors, neuropilin-1, P2X7, and CD147 in COVID-19-associated neurodegenerative diseases: inflammasome and JaK inhibitors as potential promising therapies. *Cell Mol Biol Lett*. 2022;27(1):1-21.
13. Chen ET, Thornton JT, Mulchi Jr C. Early forming a hummingbird-like hovering neural network circuitry pattern with reentrant spatiotemporal energy-sensory orientation privileged to avoid "Epilepsy" based on a biomimetic acetylcholinesterase memcapacitor prosthesis. *Sensors & Transducers*. 2015;191(8):84-99.
14. Mishra A, Goel RK. Psychoneurochemical investigations to reveal neurobiology of memory deficit in epilepsy. *Neurochem Res*. 2013;38(12):2503-2515.
15. Arend J, Kegler A, Caprara ALF, et al. Depressive, inflammatory, and metabolic factors associated with cognitive impairment in patients with epilepsy. *Epilepsy Behav*. 2018;86:49-57.
16. Akyuz E, Polat AK, Eroglu E, Kullu I, Angelopoulou E, Paudel YN. Revisiting the role of neurotransmitters in epilepsy: An updated review. *Life Sci*. 2021;265:118826.
17. Li X, Wang Q, Wu D, et al. The effect of a novel anticonvulsant chemical Q808 on gut microbiota and hippocampus neurotransmitters in pentylenetetrazole-induced seizures in rats. *BMC Neurosci*. 2022;23(1):7. doi:10.1186/s12868-022-00690-3
18. Strandwitz P. Neurotransmitter modulation by the gut microbiota. *Brain Res*. 2018;1693:128-133.