Pharmacogn J. 2023; 15(1): 150-153
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Analysis of The Effect of Leptin, AMPK, Adiponectin, and NPY Markers on Changes in Body Weight of Childhood Epileptic Using Valproic Acid Monotherapy

Wardah Rahmatul Islamiyah[1,2], Nasronudin[3,4,*], Abdulloh Machin[2], Iin Ernawati[5], Yunita Dwi Tanti[6], Nur Jaya[7], Farah Medina[8], Fathia Faza Rahmadanita[9], Paulus Sugianto[2]

ABSTRACT

Introduction: Epilepsy is a neurological disorder that occurs due to abnormal neurons in the brain and an imbalance between excitation and inhibition in the central nervous system. The first line of OAE in children is VPA (Valproate Acid). However, long-term use can cause weight gain with a frequency of 10-70%. The underlying mechanism of weight gain in patients remains unclear. **Purpose**: This study aimed to analyze the relationship between levels of biomarkers AMPK, NPY, Leptin, and Adiponectin on changes in body weight in patients with epileptic seizures using VPA monotherapy. **Method**: This study is an observational cohort design. Data collection in April-June 2019. Inclusion criteria were children aged 2-10 years who used VPA requirement less than two years, not taking any drugs that affect body weight, not diagnose systemic lupus, nephrotic syndrome, and diabetes mellitus. Bodyweight and all biomarkers measurement on subjects who came to the clinic at the time of study and at least after one month of taking VPA. A total of 17 subjects participated in this study. **Result**: The results of the statistical multivariate analysis test of VPA dosage on changes in body weight and biomarker levels found that Leptin, AMPK, Adiponectin did not significantly increase in body weight ($p>0.05$), but NPY significant increase in body weight ($p<0.05$). **Conclusion**: NPY is the most potent for appetite enhancing, preferential effect on carbohydrate intake, weight regulation, energy storage, and expenditure. Increase production of NPY, there is an increase in energy intake and then increases fat storage and body weight.

Key words: Weight gain, Leptin, NPY, AMPK, Adiponectin, Valproic acid, Epilepsy, Childhood.

Wardah Rahmatul Islamiyah[1,2], Nasronudin[3,4,*], Abdulloh Machin[2], Iin Ernawati[5], Yunita Dwi Tanti[6], Nur Jaya[7], Farah Medina[8], Fathia Faza Rahmadanita[9], Paulus Sugianto[2]

[1]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, INDONESIA.

[2]Department of Neurology, Faculty of Medicine, Universitas Airlangga, Surabaya, INDONESIA.

[3]Universitas Airlangga Hospital, Surabaya, INDONESIA.

[4]Departement of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, Surabaya, INDONESIA.

[5]Akademi Farmasi Surabaya, INDONESIA.

[6]Ngudi Waluyo Hospital Wlingi Blitar, INDONESIA.

[7]Budi Agung Hospital, Palu, INDONESIA.

[8]Oncology Hospital, Surabaya, INDONESIA.

[9]Faculty of Medicine and Health Science Maulana Malik Ibrahim State Islamic University Malang, Malang, INDONESIA.

Correspondence

Nasronudin

Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, Surabaya, INDONESIA.

Email: nasronudindr@yahoo.com ; nasronudin@fk.unair.ac.id

History

- Submission Date: 24-08-2022;
- Review completed: 21-12-2022;
- Accepted Date: 03-01-2023.

DOI : 10.5530/pj.2023.15.21

Article Available online

http://www.phcogj.com/v15/i6

INTRODUCTION

Epilepsy is a chronic disease of the brain characterized by recurrent epileptic seizures (2 times or more), in which involuntary movements involving part (partial) or the whole body are generalized and often accompanied by loss of consciousness and control of gastrointestinal and urinary tract functions. Epileptic seizures occur due to intermittent brain function disorders. Epileptic is abnormal and excessive electrical discharges in neurons paroxysmal and caused by various etiologies.[1] The incidence of epilepsy occurs with a prevalence of 49 cases per 100,000 population in low-middle-income countries. In developing countries, about 70% of children and adults with epilepsy successfully control seizures with antiepileptic drugs (OAEs) and can be discontinued without relapse or recurrence after 2-5 years of treatment.[2]

Epilepsy treatment takes a long time. Pharmacological therapy for older epilepsy such as Valproic Acid (VPA), Phenytoin, Carbamazepine, Phenobarbital is an antiepileptic drug commonly used as the first line for seizures in children and adults and is cheaper.[3] The use of AED in children often uses valproic acid. VPA is a broad spectrum OAE and is the only OAE available in syrup in Indonesia, making it an easy choice for patients to consume. VPA works by increasing GABA levels in the brain, inhibiting GABA transaminase, thereby inhibiting GABA degradation.[4] VPA has some side effects including digestive problems, prolongation of prothrombin time, hyperammonemia, thrombocytopenia, encephalopathy, alopecia, headaches, tremors, and the appearance of allergic reactions such as rashes.[5,6] The side effect reported in patients receiving VPA therapy is weight gain. The percentage side effect of weight gain is between 10%-70%.[7] This study aims to determine relationships between several markers that theoretically have affect fat and carbohydrate metabolism. This study aims to determine biomarkers such as leptin, AMPK, adiponectin, and NPY on changes in body weight in childhood epileptic patients using VPA.

MATERIAL AND METHOD

This study is a prospective observational study. The subjects of this study were pediatric patients aged 2-17 years who were diagnosed with epileptic seizures and used VPA monotherapy for two years at the IRJ Neurology Polyclinic RSUA and IRJ RSUD Ngudi Waluyo Blitar.

Inclusion Criteria:

1. Pediatric patients diagnosed with seizures who used VPA monotherapy regimen for two years

2. Patients with complete data (demographic data, drug therapy data)

3. Patients who have agreed to be involved as research subjects and have signed an informed consent

Cite this article: Islamiyah WR, Nasronudin, Machin A, Ernawati I, Tanti YD, Jaya N, et al. Analysis of The Effect of Leptin, AMPK, Adiponectin, and NPY Markers on Changes in Body Weight of Childhood Epileptic Using Valproic Acid Monotherapy. Pharmacogn J. 2023;15(1):. 150-153

Exclusion Criteria:

1. Pediatric patients with a history of diabetes mellitus and nephrotic syndrome

2. Pediatric patients using other drugs that can affect levels (corticosteroids, NSAIDs, appetite-enhancing drugs).

3. Patients who are not willing to have blood drawn at the appointed time

4. Patients who stop taking valproic acid either on the advice of a doctor or of their own accord

Data collection process

Patients who meet the criteria and their families are willing to sign the informed consent and as research subjects. Data collection such as regimentation of valproic acid monotherapy, type, frequency, intensity, and duration of seizures, other patient's demographic using medical records. Patients who met the inclusion criteria were drawn blood at pre and post (1 month after first data collection). Take 3-5 ml of whole blood from a vein and put it in an SST tube (serum separation tube), without the addition of an anticoagulant. Whole blood, then incubated for 20 minutes at room temperature and then centrifuged (1000 rpm for 5 minutes) to obtain serum. Analysis of biomarkers in serum using ELISA (Enzyme-linked immunosorbent assay)

Data analysis

The normality of data using SPSS version 23, namely the Shapiro Wilk statistic ($p>0.05$). Statistical analysis for data using Pearson's test (for correlation) and Mann Whitney (for difference test) with $p < 0.05$ for significant different.

RESULT

This study was conducted at the outpatient unit (IRJ) Neurology and Pediatrics at the Airlangga University Hospital (RSUA) and the IRJ Pediatric Health Sciences. RSUD Ngudi Waluyo Wlingi Blitar, in April-June 2019. This research has been certified ethically by the Ethics and Legal Committee of the RSUA based on the SK Lolok Ethics Test NO. 130/KEH/2019. Sampling technic by consecutive sampling and obtained 17 research subjects who took data on body weight, and biomarker levels such as Leptin, NPY, Adiponectin, AMPK pre and post (1 month after first data collection).

DISCUSSION

In this study, the total number of samples that met the inclusion criteria was 17 patients. Based on gender, there were 9 male patients (52.9%) and 8 female patients (47.1%) (Table 1). The average daily total dose consumed was 572 mg. from the study result, a total of 12 patients experienced weight gain and 5 patients experienced weight loss. The average weight change was a weight gain of 0.82 kg for one month. The mechanism underlying the weight gain in epilepsy patients taking the OAE (antiepileptic drug) valproic acid is unclear. Testing the levels of several biomarkers is used to find the basis for weight gain. This study uses adiponectin markers based on the hypothesis that the occurrence of hypothalamic dysregulation can increase appetite effects and affect adipose tissue, adipokines and trigger hyperinsulinemia, fatty acid metabolism disorders, insulin resistance, and genetic susceptibility.[8]

Of the four biomarkers observed, only the NPY biomarker was statistically significant in influencing changes in patient weight and had a positive correlation (Table 3). NPY is the most potent appetite-enhancing polypeptide. NPY has a preferential effect on carbohydrate intake, weight regulation, energy storage, and expenditure. One study by Baranowska *et al.*[8] showed that NPY levels increased earlier than leptin levels at the beginning of the obesity period. NPY can increase food intake, limit energy expenditure and decrease thermogenesis,

Table 1: Demographic characteristics.

Characteristic	Total (n=17)		
Variable	**N**	**%**	**Average ± Deviation standard**
Gender			
Male	9	52,9	
Female	8	47,1	
Age			
Young child (2-6 years)	5	29,4	10 ± 4,4
Child (6-12 years)	6	35,3	
Adolescent (12-17 years)	6	35,3	
Puberty status			
puberty	6	35,3	
No puberty	11	64,7	
Duration medication (months)			
≤ 6 months	7	41,8	12 ± 9,2
> 6 months	10	58,8	
Totale dosage/day (mg)	572 ± 316,8 (130 – 1000 mg)		
Drug dosage form			
Syrup	11	64,7	
Tablet	6	35,3	

in brown fat tissue, facilitating fat storage in white tissue through insulin activity.[10] Valproic acid (VPA) has multiple effects on cellular signaling cascades leading to various changes in gene expression. The molecular mechanism by which long-term VPA treatment increases NPY expression in the thalamus may involve epigenetic mechanisms. VPA has an inhibitory effect on HDAC (Hitondeactylase) and promotes the expression of the Brain-Derived Neurotropic Factor (BDNF) gene (a transcriptional marker for the regulation of m-RNA from NPY biomarker). Hormonal signals, particularly leptin, insulin, and glycerin affect NPY secretion. Through the Y1 and Y5 responses, NPY activates laterally. Hypothalamus Area (LHA) is the center of hunger and stimulation in this area can inhibit pro-opiomelanocortin (POMC) (the center of satiety). Besides increasing appetite, NPY has the function of regulating teratogenesis and energy intake. With an increase in NPY, there is an increase in energy intake then increases fat storage and causes an increase in body weight.[11,12]

The next biomarker to be observed was adiponectin. Adipose tissue is an endocrine organ (secretes many biologically active proteins namely adipokines and adipocytokines). Adipocytokines biomarkers such as leptin, tumor necrosis factor (TNF-α), plasminogen activator inhibitor type-1 (PAI-1), adipokines, resistin, and adiponectin.[13-15] Adiponectin is a role biomarker in the regulation of glucose and fatty acid catabolism in the body.[16,17] Adiponectin will inhibit the regulation of peroxisome proliferator-activated receptor alpha (PPAR-α) which is an important transcription factor in fat regulation, if adiponectin inhibited there will be a decrease in acetyl CoA oxidase and uncoupled protein (UCP) thereby increasing FFA oxidation and energy expenditure which leads to increased fatty acids and hyperinsulinemia.[18] In this study, the average pre-adiponectin level was 20700ng/ml and the average post-adiponectin level was 22535ng/ml. From the result of multivariate analysis and the correlation known that adiponectin levels were not statistically significant and had a negative correlation with changes in body weight (Table 3). The study from Swabic and Havel[19] known that several factors such as physical activity, patient diet, inflammation, and oxidative stress that affect the adiponectin levels.

The third biomarker is AMPK. AMPK is a heteromeric protein kinase complex activated by a low energy state and restores energy balance.[20-25] In this study, AMPK markers were not significantly different.

Table 2: Results of measurement of body weight and biomarker levels.

Subject	Body weight (kg)		Leptin (ng/ml)		NPY (ng/ml)		AMPK (ng/ml)		Adiponectin (ng/ml)		Body weight changes
	pre	post	pre	post	pre	post	pre	post	Pre	post	
1	23.5	25	0.340	0.607	0.3708	0.4543	16.322	12.291	13471	11143	(+) 1.5
2	47	50	0.534	1.345	0.301	0.3593	3.502	10.932	5629	5381	(+) 3
3	57	63	4.229	5.307	0.346	0.396	15.198	8.944	3198	2384	(+) 6
4	26	27	4.326	6.922	0.432	0.437	7.565	7.651	23309	18677	(+) 6
5	32.5	37	19.595	11.50	0.3427	0.536	9.614	4.937	6753	18012	(+) 4.5
6	33.5	36	12.503	13.443	0.4484	0.4727	10.806	7.429	22154	8444	(+) 1.5
7	33	34.5	7.18	13.3	0.306	0.320	15.231	12.291	35309	25541	(+) 1.5
8	24	25.5	4.806	4.630	0.364	0.344	10.327	11.427	44104	20950	(+) 1
9	16	17	2.16	3.516	0.3119	0.3638	13.797	13.219	10880	67800	+1
10	12	13	3.601	2.972	0.4339	0.5419	26.823	21.431	16804	14092	(-) 2
11	36	35	13.229	11.469	0.3965	0.3793	18.612	12.883	4143	18342	(-) 1
12	47	45	29.29	34.882	0.3369	0.2817	10.923	18.448	45148	4896	(-) 2
13	12.5	13	10.037	12.914	0.4081	0.3793	17.424	12.370	30512	67800	(+) 0.5
14	24	23	0.534	0.445	0.4748	0.3467	21.529	22.177	36007	41599	(-) 1
15	31	29	35.474	72.397	0.4727	0.3064	17.197	16.393	17603	22439	(-) 2
16	42	43	39.626	29.331	0.2475	0.3477	17.888	4.710	23407	12581	(+) 1
17	41	42	10.999	5.958	0.4931	0.4322	27.682	16.937	23407	23016	(+) 1
Min	12	13	0.340	0.445	0.2475	0.2817	3.502	4.710	4143	2384	
Max	60	63	39.626	72.397	0.4931	0.5419	27.682	22.177	45148	67800	
Average	32.8	33.9	11.67	13.58	0.3839	0.3941	15.3	12.6	20784	22535	
Deviation Standart	14.24	14.92	12.34	17.89	0.07378	0.0751	6.4	5.2	13497	19.423	

Note: kg:kilograms; NPY: Neuropeptide Y, AMPK: AMP-activated protein kinase; ng/ml: nanograms/mililiters; (+): there is weight gain/increase; (-): there is weight loss

Table 3: Correlation and multivariate test results of valproic acid dosage on changes in body weight and changes in biomarker levels.

Biomarker	p-value	Correlation (r)
Leptin	0.879	- 0.17
AMPK	0.838	- 0.343
Adinopektin	0.707	- 0.100
NPY	0.038	+ 0.673

Note: NPY: Neuropeptide Y, AMPK: AMP-activated protein kinase

CONCLUSION

Of the four biomarkers observed, only the NPY biomarker was statistically significant in influencing changes in patient weight and had a positive correlation. Valproic acid (VPA) has multiple effects on cellular signaling cascades leading to various changes in gene expression. NPY increasing appetite, NPY has another function of regulating teratogenesis and energy intake. With the increase of NPY, there is an increase in energy intake then increases fat storage and causes an increase in body weight.

CONFLICTS OF INTEREST

The authors declares that there is no conflicts of interest.

REFERENCES

1. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, *et al.* ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475-82.
2. WHO. Epilepsy. Available at: http://www.who.int/news-room/factsheets/detail/epilepsy accessed on 18 January 2019.
3. Lowenstein DH. Seizures and Epilepsy. In: Hauser, SL, et al. Harrison's Neurology in Clinical Medicine, 4th edition. New York: McGraw-Hill Education. 2017;297-322.
4. Nageshwaran S, Ledingham D, Wilson HC. Drugs in Neurology. Oxford University Press. 2017;619-20.
5. Gerstner T, Bell N, Konig S. Oral valproic acid for epilepsy – long-term experience in therapy and side effects. Exp Opin Pharmacotherapy. 2008;9(2):285-92.
6. Nanau RM, Neuman MG. Adverse drug reactions induced by valproic acid. Clin Biochem. 2013;46(15):1323-38.
7. Morrell MJ, Isojärvi J, Taylor AE, Dam M, Ayala R, Gomez G, *et al.* Higher androgens and weight gain with valproate compared with lamotrigine for epilepsy. Epilepsy Res. 2003;54(2-3):189-99.
8. Verrotti A, Manco R, Agostinelli S, Coppola G, Chiarelli F. The metabolic syndrome in overweight epileptic patients treated with valproic acid. Epilepsia. 2010;51(2):268-73.
9. Baranowska B, Wolinska-Witort E, Martynska M, Chmielowska M, Baranowska-Bik A. Plasma orexin A, orexin B, leptin, neuropeptide Y (NPY) and insulin in obese women. Neuro Endocrinol Lett. 2005;26(4):293-6.
10. Tokgoz H, Aydin K, Oran B, Kiyici A. Plasma leptin, neuropeptide Y, ghrelin, and adiponectin levels and carotid artery intima media thickness in epileptic children treated with valproate. Childs Nerv Syst. 2012;28(7):1049-53.
11. Arora S, Anubhuti. Role of neuropeptides in appetite regulation and obesity--a review. Neuropeptides. 2006;40(6):375-401.
12. Wysokinski A, Kloszewska I. Mechanisms of increased appetite and weight gain induced by psychotropic medications. J Adv Clin Pharmacol. 2014;1:12-33.
13. Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: more than just another fat cell hormone? Diabetes Care. 2003;26(8):2442-50.
14. Vendrell J, Broch M, Vilarrasa N, Molina A, Gómez JM, Gutiérrez C, *et al.* Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. Obes Res. 2004;12(6):962-71.

15. Gil-Campos M, Cañete RR, Gil A. Adiponectin, the missing link in insulin resistance and obesity. Clin Nutr. 2004;23(5):963-74.

16. Yadav A, Kataria MA, Saini V, Yadav A. Role of leptin and adiponectin in insulin resistance. Clin Chim Acta. 2013;417:80-4.

17. Nigro E, Scudiero O, Monaco ML, Palmieri A, Mazzarella G, Costagliola C, *et al*. New insight into adiponectin role in obesity and obesity-related diseases. Biomed Res Int. 2014;1(4):658913.

18. Achari AE, Jain SK. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int J Mol Sci. 2017;18(6):1321.

19. Abdelhalim MA, Siiddiqi NJ, Alhomida AS, Al-Ayed MS. Effects of feeding periods of high cholesterol and saturated fat diet on blood biochemistry and hydroxyproline fractions in rabbits. Bioinform Biol Insights. 2008;2:95-100.

20. Harjes U, Bensaad K, Harris AL. Endothelial cell metabolism and implications for cancer therapy. Br J Cancer. 2012;107(8):1207-12.

21. Cabral-Pereira G, Sánchez-Benito D, Díaz-Rodríguez SM, Gonçalves J, Sancho C, Castellano O, *et al*. Behavioral and Molecular Effects Induced by Cannabidiol and Valproate Administration in the GASH/Sal Model of Acute Audiogenic Seizures. Front Behav Neurosci. 2021;14:612624.

22. Budiarti R, Kuntaman, Nasronudin, Suryokusumo, Khairunisa SQ. In Vitro Studies on Heme Oxygenase-1 And P24 Antigen Hiv-1 Level Afterhyperbaric Oxygen Treatmentofhiv-1 Infected on Peripheral Blood Mononuclear Cells (PBMCS). Afr J Infect Dis. 2018;12(1S):1-6.

23. Oktafiani D, Megasari NLA, Fitriana E, Nasronudin, Lusida MI, Soetjipto. Detection of Human Herpesvirus-8 Antigen in Hiv-Infected Patients In East Java, Indonesia. Afr J Infect Dis. 2018;12(2):43-6.

24. Azizah LN, Wardhani P, Arwati H. Antimalarial Activity of Ethanol Extract of Kelakai Leaves (*Stenochlaena palustris*) to Parasitemia and Splenomegaly in BALB/c Mice Infected with *Plasmodium berghei* ANKA. JUXTA. 2022;13(1):1-5.

25. Kinanthi MTA, Irmawati M, Aprilawati D. Nutritional Status Based on Four Anthropometric Indices and Associated Factors in Children between the Ages 0-2 Years Old in a Slum of Surabaya. JUXTA. 2021;12(2):90-3.

Cite this article: Islamiyah WR, Nasronudin, Machin A, Ernawati I, Tanti YD, Jaya N, et al. Analysis of The Effect of Leptin, AMPK, Adiponectin, and NPY Markers on Changes in Body Weight of Childhood Epileptic Using Valproic Acid Monotherapy. Pharmacogn J. 2023;15(1): 150-153.