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The effect of patient's characteristics on CYP2C19 polymorphisms based on weight and gender groups of epileptic seizure patients taking divalproex sodium

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***Abstract*---**Valproic acid (VPA) is one of the most commonly used antiepileptic drugs and has broad indications for various types of epilepsy. Despite its broad spectrum, one of the most widely reported side effects of VPA is weight gain. The aim of this study was to determine the effect of demographic characteristics on genetic factors of CYP2C19 polymorphism in gender and weight changes. The research method used is analytic observational with a cross sectional study design involving two different groups and compared at the same time. Statistical analysis in this study using multiple linear regression. In observational results, it was known that the type of valproic acid (regular valproic acid and extended release) and free

testosterone levels had a statistically significant effect on CYP2C19 polymorphisms ($p < 0.05$) in the body group. Meanwhile, in the gender group, it was found that female gender with a family history of obesity had a significant influence on the incidence of CYP2C19 polymorphisms, while characteristics such as age, dose, duration of therapy, type of epilepsy, free-testosterone levels, and total estradiol levels did not have a significant effect on CYP2C19 polymorphisms. Cytochrome (CYP) P450 enzymes play an important role in VPA metabolism). Differences in population, genotype, multifactor causes, and research methods affect the results obtained.

***Keywords*---**CYP2C19 polymorphism, epilepsy, body weight, gender, valproic acid.

Introduction

Valproic acid (VPA) is one of the most commonly prescribed antiepileptic drugs. This drug also has broad indications. In addition to cases of seizures and epilepsy, this drug is also used in bipolar psychiatric disorders, schizophrenia, borderline personality disorder, and migraine prophylaxis (Li et al., 2015). Valproate is available in the form of sodium/sodium valproate. This drug has broad indications including seizures and epilepsy, bipolar psychiatric disorders, schizophrenia, borderline personality disorder, and migraine prophylaxis (Li et al., 2015). Despite having a broad spectrum, one of the most widely reported side effects of VPA is weight gain (Noai et al., 2016). The consequences of weight gain are not only bad from an aesthetic point of view but increase the risk of various other medical conditions such as insulin resistance, hypertension, cardiovascular disease, hyperleptinemia, leptin resistance (LEP), or cancer incidence (Martin et al., 2010; Li et al., 2010; Li et al. al., 2015). The frequency of the incidence of weight gain after consuming VPA still varies. A study conducted by Verotti et al (2011) and El-Khatib et al (2007) reported that a significant increase in body weight (≥ 5 kg) occurred in 43.6% of women and 23.5% of men who received VPA therapy. The mechanisms of valproic acid-related alter of lipid profiles through the insulin resistance and hyperinsulinemia (Jaeri and Islamiyah, 2018).

Administration of high doses of VPA with drugs that are primarily metabolized by CYP2C19, can lead to significant drug interactions (Tan et al., 2010). VPA can inhibit the activity of CYP2C9 and CYP2C19 metabolizing enzymes in human liver microsomes. CYP2C19 inhibition may explain some of the pharmacokinetic effects of VPA with other drugs, such as phenytoin. Previous studies found an association of CYP2C19 polymorphisms with VPA, increased appetite, and testosterone metabolism (Saruwatari et al., 2010). The study by Noai et al. (2016) showed that epilepsy patients receiving VPA therapy experienced increased testosterone levels, especially in patients who started VPA therapy at the age of less than 20 years. Testosterone levels have a positive correlation with orexigenic ghrelin levels (Borgquist, et al., 2015). Differences in population, genotype, multifactor causes, and research methods affect the results obtained. In this study, the researcher wanted to find out how the influence of demographic characteristics such as age, gender, VPA dose, VPA type, and free testosterone

and total estrogen levels on CYP2C19 polymorphisms based on changes in body weight in patients taking VPA.

Methods

This research is an analytic observational study, cross-sectional study design involving two different groups and compared at the same time. Study of the association of CYP2C19 gene polymorphisms with the incidence of weight gain in epileptic patients taking Divalproic sodium. In the process of sub-analysis, the division is based on the male and female gender. This research has passed the ethical test from the ethics committee of the Airlangga University Hospital with the number 192/KEP/2020.

Population and Research Subjects

Population
The affordable population of this study was patients with epileptic seizures who received valproic acid monotherapy at the Airlangga University Hospital.

Research subject

The subjects of this study were patients with epileptic seizures who received the drug Divalproic sodium, which met the inclusion and exclusion criteria. The research subjects were then divided into two groups, namely the weight gain group and the fixed/low weight group

Inclusion and Exclusion Criteria

Inclusion criteria
a) Patients experiencing epileptic seizures and aged 18-50 years.
b) Subjects had been taking valproic acid (Divalproic sodium) monotherapy for 6 months.
c) The subject has no history of impaired liver or kidney function. Female subjects, they have regular menstrual cycles.
d) Willing to participate in research and follow all research procedures.

Exclusion Criteria
a) Exclusion criteria in this study were subjects who were pregnant,
b) Had a history of weight gain > 2.5 kg six months before starting valproic acid therapy.
c) Subjects using hormonal contraception and corticosteroid or herbal medicine were also excluded.
d) Subjects who are following a weight gain or loss program

Research Subject Taking Techniques

This research will use purposive consecutive sampling method. This method is a sample selection method that is carried out by selecting individuals who are met and meet the selection criteria, until the desired number of samples has been met.

Research Material

Research Subject Taking Techniques

This research will use a purposive consecutive sampling method. This method is a sample selection method that is carried out by selecting individuals who are met and meet the selection criteria until the desired number of samples has been met.

Research Material

1. Materials for DNA isolation consisting of lysis buffer for cell membranes (CMLB), lysis buffer for nuclear membranes (NMLB), TE buffer (10 mM Tris HCl pH 7.4 and 0.1 mM Na2EDTA), and TBE buffer 5x (54 g Tris base, 27.5 g boric acid, 20 ml 0.5 M Na2EDTA pH 8).
2. Materials for RFLP PCR examination consisting of:
   a. The forward primer used is as in Table 1.

Table 1. Forward Primer

CYP2C19*2	*Foward*	5'-CAGAGCTTGGCATATTGTATC
	Reverse	5'-GTAAACACAAAACTAGTCAATG
CYP2C10*3	*Foward*	5'-AAATTGTTTCCAATCATTTAGCT
	Reverse	5'-ACTTCAGGGCTTGGTCAATA

   b. Whole blood of research subjects (in EDTA tube) as much as 3 ml.
   c. The extraction tools are a water bath/heating block, vortex, 1.5 mL microcentrifuge rotor with speeds of 800 xg and 20,000 xg, and a timer (timer).
   d. The amplification tool is a Veriti thermal cycler/GeneAmp PCR system 2400.
   e. The detection tools are OHAUSS analytical balance, gel electrophoresis device, and gel documentation XR.
   3. Materials or kits for checking free testosterone levels with EIA
   4. Materials or kits for checking total estrogen levels with ECLIA

Statistical analysis

Statistical analysis of this study used multiple linear regression statistical analysis using SPSS 25 software.

Result

Table 2. Patient's Characteristics

Patient's Charactheristic	Total (N)	Persentage (%)
Age (years)		
18-20	9	22.5
21-30	6	15.0
31-40	6	15.0
41-50	18	45.0

51-60	1	2.5
Gender		
Male	17	42.5
Female	23	57.5
Valproic Acid Type		
Sodium divalproex	20	50.0
Sodium divalproex ER (*Extended Release*)	20	50.0
Valproic acid Dose (mg per day)		
750 – 1500	8	20
<750	32	80
Duration therapy (month)		
<12	8	20
12-24	9	22.5
25-36	10	25
37-48	1	2.5
>48	12	30
Epilepsy type		
General	25	62.5
Focal	14	35.0
Absance	1	2.5
Menstrual Disorder		
Yes	2	20
No (Male)	32	80
Family history of obesity		
Presence	28	70.0
No	12	30.0
Freetestosterone Level		
Normal	31	7.5
Increase	1	2.5
Decrease	8	20
Total Estradiol Level		
Normal	5	12.5
Increase	34	85
Decrease	1	2.5

Table 3. Effect of Patient's Characteristics on Polymorphism CYP2C19 in the weight group

Multiple Linear Regression Test	Weight Gain Group			Weight Loss Group		
	B	SE	*P*-Value	B	SE	*P*-Value
Age	-0.007	0.083	0.933	0.077	0.103	0.476
Gender	0.235	0.399	0.573	-0.282	0.267	0.321
VPA type	0.525	0.831	0.545	-1.075	0.419	0.033
VPA dose (mg per day)	0.030	0.150	0.847	0.144	0.106	0.212
Duration of Therapy (month)	0.001	0.019	0.978	-0.007	0.015	0.650

Epileptic type	-0.070	0.216	0.753	0.114	0.191	0.568
Menstrual disorder	-0.093	0.248	0.718	0.244	0.191	0.237
Family history of obesity	0.135	0.137	0.354	0.117	0.089	0.224
Freetestosteron Level	-0.054	0.153	0.734	-0.415	0.157	0.029
Total Estradiol Level	-0.194	0.336	0.580	-0.116	0.198	0.574

B: Regression coefficient; **SE:** Standard Error

Table 4. The Effect of Patient's Characteristics on Polymorphism CYP2C19 in the Gender Group

Multiple Linear Regression Test	Male			Female		
	B	SE	*P-Value*	B	SE	*P-Value*
Age	0.063	0.059	0.326	0.022	0.069	0.759
Gender						
VPA Type	0.144	0.556	0.803	-0.600	0.527	0.277
VPA dose (mg per day)	0.081	0.129	0.549	0.058	0.126	0.653
Duration of Therapy (months)	-0.006	0.019	0.744	-0.014	0.014	0.336
Epileptic type	0.028	0.263	0.917	0.077	0.155	0.629
Menstrual Disorder				0.195	0.163	0.255
Family history of obesity	-0.019	0.104	0.860	0.281	0.117	0.033
Freetestosteron Level	0.037	0.129	0.781	-0.158	0.316	0.626
Total Estradiol Level	-0.256	0.303	0.426	-0.171	0.280	0.554

Table 5. The Effect of Obesity History on Polymorphism CYP2C19 in the Gender Group

Partial regression	Male				Female			
	R square	B	SE	P value	R square	B	SE	p
History obesity	0.018	-0.039	0.075	0.612	0.288	0.250	0.086	0.008

Discussion

This study is an observational analytic study that aims to determine the influence of demographic characteristics on genetics, especially the presence of polymorphisms of the CYP2C19 metabolizing enzyme, which was carried out in two weight groups of patients taking the antiepileptic drug Divalproex Sodium. The research subjects obtained in this study were 40 patients who used Divalproex Sodium with a maximum dose of <750 mg and the majority were female (57.5%) (Table 1). The valproic acid used in this study was the regular Divalproex Sodium and the ER (*Extended Release*) VPA formulation with delayed release. VPA is rapidly absorbed from the gastrointestinal tract with between 1-4 hours (tablets taken orally). VPA is mostly metabolized in the liver, and only a

small amount is not metabolized in the liver but is directly excreted in the urine. In ER formulations, the dosing interval is usually extended to minimize the frequency of dosing (Bialer, 2007; Leppik and Hovinga, 2013; Zhang et al., 2021). Compared with the standard formulation, once-daily Divalproate sodium Extended Release (ER) significantly stabilizes serum levels without fluctuations in peak drug levels, reduces dosing frequency, dose flexibility, improves patient compliance, satisfaction and quality of life (Genton, 2005; Zhang et al., 2021).

CYP2C19 is an enzyme that functions in various drug metabolism and can be genetically polymorphic. This enzyme can metabolize various types of drugs such as psychotropic drugs, such as antidepressants (selective serotonin reuptake inhibitors, tricyclic antidepressants), benzodiazepines, and antiepileptics (valproate). Polymorphism will affect the ability of genetic CYP2C19 enzyme activity. If the function is decreased, it is called a poor metabolizer CYP2C19 gene polymorphism (Stingl et al., 2021). Many studies have reported that the cytochrome P450 enzyme (CYP) plays an important role in VPA metabolism. In patients with the CYP2C19*2 allele (an enzyme essential for VPA metabolism), higher VPA doses are required to achieve a VPA concentration > 50 g/ml (Zhu et al., 2017). A study conducted by Jiang et al (2003) stated that patients receiving VPA and having genotypes CYP2C19*2/*3 and CYP2C19*3 affect the therapeutic level of VPA (Tan et al., 2010). Research related to the prevalence of CYP2C19 polymorphism in Indonesia known as CYP2C19*2, it was observed that 77 (46.4%) of the 166 patients had the homozygous wild-type allele (*1/*1) and in total, 24 (14.5%) had the homozygous mutated allele (*2/*2); a total of 65 (39.2%) had the heterozygous allele (*1/*2) (Miftahussuhurr et al., 2021).

Based on tables 2 and 3, statistical analysis using a multiple linear regression test, where this test looks for the influence of each patient's characteristic variable on the CYP2C19 polymorphism. The subjects of this study were divided into 2 groups, namely the group of weight gain and loss and the gender of men and women. In the weight group, it is known that the VPA type variable has a value (Regression Coefficient (B) of -1.075. In the VPA type variable, the p-value is 0.033 (p-value <) which means that there is an effect of the VPA type variable on the CYP2C19 gene polymorphisms in particular on weight loss. This study is in line with previous studies if the increase in body weight appears to be less large in the extended-release (ER) formulation of VPA (Smith, et al., 2004; Martin et al., 2009). A higher serum level and higher body weight are associated with greater weight gain (Bowden and Singh, 2005). Divalproex sodium has various formulas namely traditional, enteric-coated, delayed-release tablet (Depakote), sprinkle capsule, and extended-release (Depakote ER). Divalproex ER sodium is a tablet-controlled-release hydrophilic polymer matrix that will slowly release the drug in the stomach, small intestine, and large intestine within 18 to 24 hours. VPA ER type is more no risk of causing weight gain (Smith et al., 2004).

The next variable is related to Free-Testosterone Levels with a value (Regression Coefficient (B) of -0.415. This value indicates a negative (opposite direction) effect between the Free-Testosterone variable and the polymorphism variable. The rate of catalysis of testosterone into estrogen by the CYP2C19 enzyme will affect the free testosterone level. The faster the process is catalysis (ultra-metabolizer) will cause free testosterone levels to be depleted more quickly. Meanwhile, a slow

catalysis process (poor metabolizer) will cause an increase in free testosterone levels. This condition can explain the negative influence between the free testosterone variable and the CYP2C19 gene polymorphism. In the male sex, the increase in free testosterone levels will have a weight loss effect, while in women it will increase body weight. This means that if the Free-Testosterone variable increases by 1%, the levels of the CYP2C19 polymorphism will also decrease by as much as are 0.415. And the Free-Testosterone variable obtained a p-value of 0.029 (p-value < 0,05) which means that there is an influence between the Free-Testosterone variable on polymorphism levels. Other demographic characteristics such as age, gender, VPA dose, duration of seizures, type of epilepsy, menstrual disorders, history of obesity, and free estradiol levels did not have a significant effect on the CYP 2C19 polymorphism.

In the female gender group, there is an influence of the Family History of Obesity variable on polymorphism with a value (Regression Coefficient (B) of 0.281. This value indicates a positive (unidirectional) effect between the family history of obesity variable and the CYP2C19 polymorphism variable. The family history of obesity variable obtained a p-value of 0.033 (p-value < 0,05) which means that there is an influence between the family history of obesity variable on the levels of CYP2C19 polymorphism in the female sex group. When sub-analysis was performed with partial regression in table 4, there was an effect of family history of obesity on CYP2C19 in the female group of 28% (R square 0.288) and obtained a p-value of 0.008 (p-value <) which means the effect of family history of obesity on CYP2C19 in the female group is significant. This is in accordance with the research of Anita et al in 2019 which stated that there exists “familial aggregation” in familial correlations of obese/overweight state during early days of life whereby daughters are more likely to be inflicted upon by parental indices of adiposity than sons. Consistent with these lines, a positive history in family for obesity cases has been highlighted in the present study to be one of the major diagnostic risk factors for the onset of weight gain, equally suggested by many other studies (Mangla et al., 2019). In obese patients, inflammation of adipose tissue occurs and causes insulin resistance and an increase in aromatase expression (in this case the CYP2C19 gene polymorphism) which converts testosterone to estradiol. Estradiol in adipose provides negative feedback in the form of decreased pituitary gonadotropin secretion. This increases the metabolic ability of the CYP2C19 enzyme. Based on table 3, age, VPA dose, type of VPA, duration of seizures, type of epilepsy, menstrual disorders, free testosterone levels, and total estrogen levels did not affect the CYP2C19 gene polymorphism in both male and female sex groups.

Conclusion

In this study, the results showed that there was an effect of VPA type and free testosterone levels on CYP2C19 gene polymorphisms in the fixed or decreased body weight group. ER type VPA is thought to be less likely to cause side effects of weight gain. Free testosterone levels affect the work of the CYP2C19 enzyme in catalyzing free testosterone. The effect of family history of obesity on CYP2C19 gene polymorphisms in the female sex group was also found. A history of obesity in women will affect the work of the aromatase enzyme CYP2C19.

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