Original Article

Side effects of long-term antiepileptic drugs on renal tubules of Indonesian children

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Abstract

Background Long-term treatment with antiepileptic drugs such as valproic acid (VPA) and carbamazepine (CBZ) may disrupt renal tubular function. Urinary N-acetyl-beta-D-glucosaminidase (NAG) may reflect tubular function and may be useful in detecting early-stage tubular injury. To date, no study has investigated the toxic effect of VPA and CBZ on renal tubules using urinary NAG in Indonesian children.

Objectives To determine the toxicity of long-term VPA and/or CBZ treatment on renal tubules in children with epilepsy by measuring urinary NAG index (iNAG).

Methods This cross-sectional study was conducted from January to March 2015 at Cipto Mangunkusumo Hospital and Anakku Clinic Pondok Pinang, Jakarta. We included children aged 3 to 16 years with epilepsy on VPA (n=36), CBZ (n=14), or VPA-CBZ combination (n=14) therapy. We measured urinary levels of creatinine and NAG. The urinary NAG reference value was obtained from age-matched healthy controls (n=30). To eliminate diurnal variations in NAG, iNAG was calculated by dividing urinary NAG by urinary creatinine. A urinary iNAG of more than 2 standard deviations above the mean for healthy children was considered elevated.

Results Mean urinary iNAG values for the control, VPA, CBZ, and combination groups were 3.01, 5.9, 4.07, and 6.9 U/g, respectively. All treated groups had higher mean urinary iNAG values compared to the control group. Urinary iNAG was increased in 11/36 children on VPA, 2/14 children on CBZ, and 9/14 children on combination therapy.

Conclusion Long-term VPA use may impair renal tubular function, as shown by the increased urinary iNAG. Combination therapy increases damage in the renal tubules.[Paediatr Indones. 2018;58:84-9; doi: http://dx.doi.org/10.14238/pi58.1.2018.84-9].

Keywords: epilepsy; valproic acid; carbamazepine; urinary iNAG; renal tubular injury

The kidneys perform essential functions of excretion and hormone production.[1] One of the excretory functions is to eliminate foreign chemicals (xenobiotics), such as drugs and their metabolites.[2] A long-term use of drug could be harmful for the kidney. Nephrotoxic agents account for approximately 20% cases of acute kidney injury (AKI).[3,4] The typical course of AKI involves multiple mechanisms including hemodynamic changes in the glomeruli, tubular cell toxicity, and interstitial nephritis. Despite the injury is usually preventable and reversible, the nephrotoxic agents should always be used cautiously.[5,6]

The glomerulus and renal tubules are responsible for excretory function. A number of different laboratory examinations are available to evaluate this function.[1] Serum creatinine is the most frequently used parameter to detect abnormalities of glomerular function; however, it is not specific and may not be helpful to detect signs of early AKI.[7] Although the decline of glomerular performance is due to the tubular injury, and vice versa, serum creatinine cannot

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be used to evaluate tubular function.[1] Therefore, there is an immediate need to find other biomarkers to detect kidney injury. Recently, studies have reported urinary N-acetyl-beta glucosaminidase (NAG) as a promising marker to detect the kidney's excretory function. The urinary level of NAG reflects the excretory performance of renal tubules, and increased urinary NAG indicates injury to the renal tubules.[8,9] Unfortunately, no universal reference value for normal urinary NAG values are currently available; previous studies have determined their own reference values according to the reagent or substrate used.

Epilepsy is a chronic clinical condition that requires long-term pharmacological treatment and may lead to a major morbidity. The incidence of epilepsy is approximately five to seven cases per 10,000 children per year. Epilepsy affects five in every 1,000 children aged zero to 15 years.[10,11] The high prevalence of epilepsy may result in the long-term use of antiepileptic drugs, such as valproic acid (VPA) and carbamazepine (CBZ). Over the last two decades, a number of studies have shown that VPA and CBZ are nephrotoxic. Although the incidence is unknown, previous reports have demonstrated that renal tubular injury in patients treated with VPA or CBZ is associated with high urinary NAG index (iNAG). This index calculates the ratio of NAG per urinary creatinine (U/g creatinine).[12-18] Since antiepileptic drug toxicity has a high interethnic variability,[19] it is necessary to determine this association in various ethnic groups.

The urinary iNAG of Indonesian children receiving antiepileptic drugs has not been previously documented. Observing the increase of urinary iNAG, instead of serum creatinine, will help clinicians to be recognize renal tubular injury at an earlier stage. This study aims to measure urinary iNAG as a marker of renal injury in Indonesian children with epilepsy who receive long-term treatment with VPA and CBZ.

Methods

This cross-sectional study was conducted from January to March 2015 at the Neurology Outpatient Clinic of the Department of Child Health, Cipto Mangunkusumo Hospital, and Anakku Clinic, Pondok Pinang, Jakarta, Indonesia. The subjects of this study were epileptic children who received valproic acid and carbamazepine as a single or combination therapy. Required subject number was calculated using single proportion formula, which determined that minimal 30 subjects was needed in each treatment groups. We included children who have received their antiepileptic drug regimen for at least 6 months who had no previous kidney or liver disease, no signs or symptoms of urinary tract infection, and normal serum ureum and creatinine levels. Patients with short stature or hypertension were excluded. The dose of the antiepileptic drugs was determined and adjusted as needed by the patient's treating pediatric neurologist. Sixty-four children aged three to 16 years were enrolled and assigned into three groups: VPA monotherapy (n=36), CBZ monotherapy (n=14), and VPA and CBZ combination therapy (n=14). Thirty clinically healthy and well-nourished children were selected consecutively from General Pediatric Clinic of Cipto Mangunkusumo Hospital, assigned as the age-matched control group.

We measured serum ureum and creatinine levels and performed urinalysis in all subjects. We also determined urinary creatinine and NAG levels. The measurement of urinary NAG level was done at Prodia Laboratories, Jakarta, by colorimetry using the 3-cresolsulfonphthaleinyl-N-acetyl-D-glucosaminide reagent (*Roche Diagnostics*, Indianapolis, USA). To eliminate diurnal variations in urinary NAG, iNAG was calculated by dividing the urinary NAG level by the urinary creatinine level. An eleveated iNAG was defined as an iNAG level higher than +2 standard deviations (SD) of the mean iNAG in the control group. The urine samples for iNAG measurement were kept in -20°C temperature until all the samples were ready to be tested at the same time, to minimize bias associated with the reagent and assay tools.

The study protocol was approved by the Medical Research Ethics Committee of the Universitas Indonesia Medical School. We obtained informed assent and consent from each subject and his or her parent or guardian.

We used the unpaired T-test to evaluate the mean difference of iNAG between each individual treatment group and the control group. If the data did not meet the requirements for parametric testing, the Mann-Whitney test was employed. The differences in the proportion of subjects with elevated urinary

iNAG between the groups were analyzed using the Kruskal-Wallis test. P values of <0.05 were considered statistically significant.

Results

Subject characteristics are shown in **Table 1**. Mean (SD) urinary iNAG in the VPA, CBZ, VPA+CBZ, and control groups were 5.9 (4.17), 4.07 (2.36), 6.9 (2.98), and 3.01 (1.83) U/g creatinine, respectively (**Table 2**). The mean difference was statistically significant between the VPA and control groups (P=0.009) and between the VPA+CBZ and control groups (P<0.001). The difference in urinary iNAG between the CBZ and control groups did not reach statistical significance (P=0.244).

Using a cut-off level of +2SD of the control group mean (3.0+3.6 U/g creatinine), eleven out of 36 subjects in the VPA group, 2/14 subjects in the CBZ group, and 9/14 subjects in the VPA+CBZ group had elevated urinary iNAG. The difference between these three treatment groups was statistically significant (P=0.017) (**Table 3**).

Discussion

This study is the first in Indonesia to evaluate antiepileptic drug-induced renal tubular injury in children using urinary iNAG as a biomarker. Urinary iNAG is a more sensitive parameter of renal excretory function than serum ureum and creatinine to evaluate renal excretory function, since changes in the iNAG

Table 1. Subjects' characteristics

Characteristics	Group I VPA (n = 36)	Group II CBZ (n = 14)	Group III VPA+CBZ (n = 14)	Group IV Control (n = 30)
Median age (range), years	9 (4-14)	8.5 (4 - 14)	10 (3 - 16)	8 (3 - 14)
Gender				
Male	21	7	7	18
Female	15	7	7	12
Nutritional status				
Obese	14	4	3	5
Overweight	5	1	1	6
Well-nourished	15	8	8	17
Underweight	2	1	2	2
Length of therapy (range), months	16 (7-70)	28 (12-75)	20.5 (8-102)	-
Median dose of antiepileptic drugs (range), mg/kg/day				
VPA	20 (7.5-49)	-	30 (12.5-50)	-
CBZ	-	15 (10-30)	20 (10-45)	-

VPA: valproic acid; CBZ: carbamazepine

Table 2. The urinary iNAG levels in epileptic *vs.* healthy children

	VPA (n =36)	CBZ (n =14)	VPA+CBZ (n =14)	Control (n=30)
Mean iNAG (SD), U/g creatinine	5.919 (4.17)	4.07 (2.36)	6.9 (2.98)	3.01 (1.83)
P value	0.009	0.244	< 0.001	-

VPA: valproic acid; CBZ: carbamazepine; SD: standard deviation

Table 3. Subjects with elevated urinary iNAG

	VPA (n=36)	CBZ (n=14)	VPA+CBZ (n=14)	P value
Subjects with elevated iNAG	11	2	9	0.017

Cut-off level: + 2 SD control group [3.0 (SD 3.6) U/g creatinine group]

occur earlier than changes in serum ureum and creatinine levels. As iNAG is influenced by age, control subjects should be age-matched to their treated counterparts. We attempted to minimize measurement bias associated with the reagent and assay tools by performing the urinary NAG examination at the same time for all specimens.

We enrolled a total of 94 children, consisting of 53 boys and 41 girls. Twenty-six subjects were obese, 13 were overweight, 48 were well-nourished, and 7 were underweight. Obesity was most frequent in the VPA group, possibly due to a well-known side effect of the drug. Verotti et al.[20] reported higher weight gain in the first 3 months after consuming VPA is due to a multifactorial etiology. Several hypotheses explaining VPA-associated weight gain include hypothalamic dysregulation, hyperinsulinemia, insulin resistance, and genetic susceptibility.

The number of participants treated with CBZ did not meet the minimum sample requirement. Therefore, we expanded the age group from six to 12 to three to 16 years. However, the total number of subjects enrolled in the CBZ group and the VPA+CBZ group remained insufficient, at only 14 subjects in each group. The small sample size in those groups resulted in higher variability of urinary iNAG, as urinary iNAG level is influenced by age. The difficulty in recruiting subjects who received CBZ may be due to CBZ being less preferred by clinicians and patients due to its potential to cause the life-threatening side effect known as Stevens-Johnson Syndrome (SJS). Forty-percent of SJS cases are linked to CBZ, and its incidence is higher in Southeast Asians.[21-23]

We required our subjects to have taken anti-epileptic drugs for a minimum of six months, to anticipate the possibility of time dependency in the emergence of renal toxicity. In this study, the length of treatment across groups ranged from seven to 102 months. The monotherapy groups had relatively lower median doses of the respective drugs, while the combination therapy group had higher median doses for both VPA and CBZ, possibly reflecting epilepsies that are more difficult to manage. However, the median treatment duration in the VPA+CBZ group was not the highest amongst the three treatment groups, although the range was widest. This finding suggests that at least half of the patients in the combination therapy group were managed with more aggressive adjustments to their treatment regimens compared to their monotherapy counterparts.

Compared to the control group, iNAG was higher in the VPA monotherapy group and in the VPA+CBZ group. The highest iNAG value was observed in the VPA+CBZ group, indicating that renal tubular injuries are worsened by combination therapy. These findings are consistent with previous studies. Animal studies have shown that valproic acid blocks fatty acid β-oxidation in the mitochondria and stimulates peroxisome proliferation in the liver and kidney. In addition, valproic acid increases glutamine uptake and ammonia production in the renal tubules.[24] However, the mechanism occurring in the renal tubules remains unclear. In a cohort study by Verrotti et al.,[15] patients treated with VPA and CBZ had increased iNAG values after six months of therapy. Such a result was not observed in patients treated with phenobarbital. Unay et al.[17] similarly reported higher iNAG in children treated with VPA monotherapy, CBZ monotherapy, and combination therapy compared to untreated controls. However, there was no difference in iNAG between children receiving lamotrigine therapy and controls. This is supported by Mazaheri et al.,[18] who higher iNAG values in subjects using VPA and CBZ compared to healthy children and untreated epileptic patients. Three previous studies showed increased iNAG in patients receiving CBZ monotherapy compared to healthy controls.[15,17,18] In contrast, we found a slightly higher mean iNAG in the CBZ monotherapy group compared to the control group, but this difference was statistically insignificant. This could be due to the number of subjects in the CBZ group being fewer than the minimum required sample size.

Various methods are available to measure iNAG and its reference value. Consequently, it is difficult to compare and interpret iNAG from the different techniques reported. Csathy et al.[25] reported the first systematic review of various methods of iNAG measurement. This review compiled studies from 1962 to 1992 which applied various techniques, substrates/reagents, and different statistical analyses. Skalova and Chladek[26] evaluated iNAG values in 262 healthy children aged zero to eight years. They used the fluorimetry method, with creatinine expressed in units of nkat/mmol. The difficulty in converting urinary iNAG values measured using different methods and

reagents has necessitated each study to provide its own control group for reference values.

We also evaluated the proportion of subjects with increased iNAG based on a reference value of our control group mean + 2SD, yielding 3.0 (SD 3.6) U/g creatinine as our cut-off point. The proportion of subjects with elevated iNAG in the VPA+CBZ group was twofold that in the VPA group, and the proportion of subjects with elevated iNAG in the VPA group was in turn twice as high as in the CBZ group.

In a study by Korinthenberg *et al.*[12] involving 20 subjects receiving VPA, 27 subjects receiving CBZ, 9 subjects receiving ethosuximide, 8 subjects receiving phenobarbital, and 23 age-matched controls, increased iNAG was defined as the iNAG level higher than the 95th percentile of the control group iNAG values. In that study, 33% of subjects in the ethosuximide group, 20% of subjects in the VPA group, and 25% of subjects in the phenobarbital group had increased iNAG. The elevation of iNAG in the CBZ group was not statistically significant.

Similar to our study, Otsuka *et al.*[13] used +2SD urine iNAG of the control group as the cut-off point. The study by Otsuka also measured serum VPA level in subjects receiving VPA and found a serum VPA of $>60\,\mu g/mL$ in 47% of subjects and $<60\,\mu g/mL$ in 24% of subjects. In the same study, 38% subjects receiving CBZ and 25% subjects receiving combination therapy had elevated urine iNAG. Csathy *et al.*[16] used a similar cut-off point and found that 45% subjects receiving VPA, 26% subjects receiving CBZ, and 36% subjects receiving combination therapy had elevated urinary iNAG.

One of the limitations of our study was the inability to obtain age-specific baseline data on normal iNAG values due to the limited number of subjects in the control group. Therefore, we were only able to compare urinary iNAG values between each respective treatment group and the control group, and not between age groups. As we did not measure serum antiepileptic drug levels, we were unable to observe the association between the cumulative dose of the drug received and iNAG values. Moreover, our cross-sectional design did not allow the determination of the timing of the rise in urinary iNAG, so that this study is unable to inform clinicians on the optimal timing to evaluate the urinary iNAG in children receiving antiepileptic drugs.

To date, there is no recommendation on the further management of patients with elevated iNAG secondary to antiepileptic drug treatment. Nevertheless, clinicians should be more vigilant of the potential nephrotoxic effects of antiepileptic treatment, especially when prescribing combination therapy, as the consequences of elevated iNAG in subjects using VPA and CBZ can be observed only after long-term follow-up. These drugs should be given in an appropriate dose and duration to minimize the side effects.[12,18] Patients with increased iNAG should undergo further evaluation, dose adjustment, or, whenever possible, modification of therapy to favor non-nephrotoxic drugs, such as lamotrigine and levetiracetam. Such steps are important to prevent further impairment of kidney function. These patients should be closely monitored and their parents should be informed of the potential side effects of the drugs received, any alternative therapeutic regimens available, and further examinations needed. Effective communication between parents, patients, and clinicians will improve the outcomes of patients who already show increased iNAG due to antiepileptic drug treatment.

In conclusion, the mean iNAG in children with epilepsy receiving VPA is twice as high as that in healthy children. In children receiving VPA and CBZ combination, the mean iNAG is 2.3 times higher than in healthy children. Children receiving VPA and CBZ combination therapy have the highest proportion of elevated iNAG, followed by those receiving VPA monotherapy. Urinary iNAG should be monitored in children receiving VPA and CBZ to detect drug-induced nephrotoxicity. A prospective study involving periodical measurement of urinary iNAG is essential to help clinicians determine the ideal timing of urinary iNAG monitoring.

Conflict of Interest

None declared.

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