ISSN Print: 2664-8350
ISSN Online: 2664-8369
Impact Factor: RJIF 5.26
IJPN 2024; 6(1): 05-10
www.pediatricsjournal.net
Received: 05-11-2023
Accepted: 09-12-2023

Heba Refat Abdalla
Department of Pediatrics, Faculty of Medicine, Tanta University, Tanta, Egypt

Sahar Abd El Azim Abd El Aziz
Department of Pediatrics, Faculty of Medicine, Tanta University, Tanta, Egypt

Hesham Ahmed Elsrogy
Department of Clinical Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt

Hala Ibrahim Hantash
Department of Pediatrics, Faculty of Medicine, Tanta University, Tanta, Egypt

Corresponding Author:
Hala Ibrahim Hantash
Department of Pediatrics, Faculty of Medicine, Tanta University, Tanta, Egypt

Urinary N- acetyl - βeta -D- glucosaminidase (NAG) in children with epilepsy treated with sodium valproate monotherapy

Heba Refat Abdalla, Sahar Abd El Azim Abd El Aziz, Hesham Ahmed Elsrogy and Hala Ibrahim Hantash

DOI: https://doi.org/10.33545/26648350.2024.v6.i1a.60

Abstract
Background: The central nervous system (CNS) is impacted by the condition known as epilepsy. It is one of the most common neurologic diseases in children. Children (those less than the age of 16) are reported to have an incidence of epilepsy that ranges from 41/100000 to 187/100000. The purpose of this work was to measure urinary N-acetyl-Beta-D- glucosaminidase NAG excretion in children with epilepsy, treated with sodium valproate monotherapy to evaluate the impacts of sodium valproate on renal tubular functions.
Methods: This cross-sectional study was performed on 50 children with epilepsy treated with sodium valproate monotherapy for not less than six months or more, with generalized or partial seizures or partial seizure with secondary generalization and 30 children who were physically healthy and well-fed were chosen. As the age-matched control group. All patients were subjected to neuro-imaging and electroencephalogram study (MRI and EEG), routine laboratory studies and specific investigations (Urinary N-Acetyl-βeta-D-glucosaminidase enzyme assay).
Results: A substantial positive association was existed among the treatment duration and the urinary NAG index. Increased duration of treatment with sodium valproate is associated with increased urinary NAG index. (r=0.309, P=0.029). The mean urinary NAG and urinary NAG index in children with epilepsy were substantially greater than the control children. (P-value < 0.001).
Conclusion: Sodium valproate has significant time-related adverse effect on renal functions. It induces histopathological alterations and oxidative stress in tissues of kidney causing tubulointerstitial nephritis. Also, the capacity of the renal tubules for reabsorbing electrolytes, protein, glucose, and urea is being lost in cases of Fanconi syndrome produced by valproate, which is becoming more prevalent. Increased urinary NAG and urinary NAG index in epileptic children received sodium valproate treatment is an early indicator of renal impairment. There is substantial potential association among urinary NAG and treatment duration, The increase in urinary NAG excretion becomes greater as the duration increases.

Keywords: Urinary N- acetyl - βeta -D- glucosaminidase, epilepsy, sodium valproate monotherapy, renal tubular functions

Introduction

The central nervous system (CNS) is impacted by the condition known as epilepsy. It is one of the most common neurologic diseases in children [1]. Children (those less than the age of 16) are reported to have an incidence of epilepsy that ranges from 41/100000 to 187/100000 [2].

Preventing the probability of any further episodes is the fundamental goal of epilepsy therapy [3]. Pharmacotherapy is the primary strategy for treating epilepsy. For these individuals, selecting the right prescription necessitates taking into account a variety of factors, including the kind of epilepsy, patient age, the adverse reactions of the medication being used, potential interactions with other drugs the patient is taking, and whether it is possible to perform monitoring of drug level [4].

Valproate has a wide range of anticonvulsant action, however it is often used as a first-line therapy for myoclonic, absence, and tonic-clonic seizures, as well as a second-line therapy for partial epileptic seizures and spasms in infants [5]. Additionally, it has been administered via IV with efficacy for the treatment of status epilepticus [6, 7].

One of the most popular medications for children having epilepsy is sodium valproate, a wide spectrum therapy [8]. One of the most popular medications for children having epilepsy is sodium valproate, a wide spectrum therapy [9]. Renal tubular dysfunctions linked to sodium valproate treatment have been documented recently. There have been reports of proximal tubular renal syndrome brought on by valproic acid [10]. After a long duration of treatment, The functioning of the kidneys may alter as a result of sodium valproate [11].

A lysosomal hydrolase enzymes involved in the breakdown of glycosaminoglycans, glycoproteins and gangliosides is known as urinary N-acetyl-β-D-glucosaminidase (NAG). [12]. Proximal tubular cells' lysosomes contain NAG [13]. NAG is a lysosomal enzyme that is employed as a detection agent in tubulopathy in the kidneys, as is well known [14, 15]. A hydrolytic enzyme called urinary NAG has a molecule weight of around 140000 Daltons. There are 2 isoenzymes {A and B} are found in humans [16]. The A isoenzyme is found in the lysosomes of proximal tubular cells, and it has been acknowledged that a higher level of the enzyme in urine is a sign of renal tubular damage based on a number of clinical investigations [13, 17].

The aim of this work was to measure urinary excretion of NAG in children having epilepsy, treated with sodium valproate monotherapy to evaluate the impacts of sodium valproate on renal tubular functions.

Patients and Methods

This cross-sectional study was performed on 50 individuals, their age ranging between 3-16 years with epilepsy (33 male, 17 female) with epilepsy treated with sodium valproate monotherapy for at least six months or more, with generalized or partial seizures or partial seizure with secondary generalization and selected from outpatient clinic of neurology unit, pediatric department, Tanta University hospital from January 2020 to January 2021 and 30 Children who were age-matched for the control group were chosen sequentially from Tanta University Hospital's General Pediatric Clinic and divided to be clinically healthy and well-fed.

After receiving clearance from Tanta University's Ethical Committee, the research was carried out. All participants in the research have their parents' written, informed permission.

Exclusion criteria were patients with degenerative or neurometabolic diseases, receiving corticosteroids or excessive doses of vitamins, with any endocrinal diseases, brain tumours, and with cerebral palsy and with renal diseases or hypertension.

Each participant had thorough history, physical, and neurological examination, routine neuro-imaging and electroencephalogram study (MRI and EEG), routine laboratory studies including liver functions, urea, creatinine and serum sodium valproate levels and specific investigations including Urinary NAG enzyme assay, urinary NAG index, urinary creatinine and creatinine clearance.

Sample

Random urine sample was collected from each patient and control in a clean container, centrifuged for 20 minutes at a rate of 2000-3000 rpm, then collected the supernatant. The centrifuged samples were stored at -20 °C until every sample was prepared for simultaneous testing for NAG estimation, to reduce the amount of bias brought on by the reagent and assay instruments.

Estimation of urinary NAG

Estimation of urinary NAG was done by an ELISA kit with a double-antibody sandwich provided by the biokits company, catalog number 201-12-0829. Since the normal values of the NAG indexes vary on age, only certain age groups may be used to compare the results.

The kit used ELISA to assay the level of human NAG in samples. After pre-coating a monoclonal antibody enzyme well with human NAG monoclonal antibodies and incubating it, adding biotin-labeled NAG antibodies and combining them with streptavidin-HRP to create an immunological complex, NAG was added. This was followed by further incubation and wash to eliminate the uncombined enzymes.

After adding chromogen solutions A and B, the liquid's color became blue. Acid's effects caused the color to ultimately become yellow. The quantity of the human substance NAG in the sample and the color chroma were significantly associated.

Calculation

The horizontal value was the standard density, while the vertical value was the OD value. On graph paper, the standard curve was drawn, and the corresponding density was determined using the sample OD value and the sample curve, or the standard curve's straight line equation for regression with the standard density and the sample OD value was calculated, and the sample density was determined using the equation and the sample OD value.

Statistical analysis

SPSS v26 (IBM Inc., Armonk, New York, USA) was used for the statistical analysis. The unpaired Student's t-test was used to compare quantitative data across the two groups. The quantitative data were provided as mean and standard deviation (SD). The Chi-square test was used to analyze qualitative data, which were reported as frequency and percentage (%). For the purpose of identifying any association among two quantitative factors in a single group, the linear correlation coefficient (r) was utilized. Statistical significance was defined as a two-tailed P value < 0.05.

Results

No substantial variation was existed among children with epilepsy and the control children regard age, sex, levels of serum creatinine, serum urea, and liver function tests. Table 1.

Table 1: Demographic data and routine laboratory investigations of studied groups.

		Case group (n=30)	Control group (n=50)	P-value
		Mean ± SD	Mean ± SD	
Age		9.540±3.032	9.000±2.754	0.428
Gender	Male	33(66.00%)	14(46.67%)	0.089

	Female	17(34.00%)	16(53.33%)	
Kidney function tests				
Urea (mg/dl)		29.860±9.067	28.267±8.835	0.445
Creatinine (mg/dl)		0.712±0.144	0.660±0.127	0.108
Liver function tests				
SGOT (U/L)		23.280±9.424	25.567±7.347	0.259
SGPT (U/L)		23.140±7.913	24.300±5.664	0.485
Serum valproate level (microg/mL)		63.960±20.64		
Duration of valproate level (months)		33.140±14.12		

Data are presented as mean ± SD or frequency (%). SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase

The mean urinary NAG and urinary NAG index in children with epilepsy were substantially greater than the control children. (p-value < 0.001). No substantial variation was existed among the children with epilepsy and the control children regard creatinine clearance and urinary creatinine.

Table 2: The mean urinary NAG level, creatinine clearance, urinary creatinine and the mean urinary NAG index in children with epilepsy and the control children.

	Case group (n=30)	Control group (n=50)	P-value
Urinary NAG (ng/ml)	6.182±1.331	4.309±1.091	<0.001*
Creatinine clearance (ml/min)	109.575±17.972	109.238±10.302	0.926
Urinary creatinine (mmol/l)	9.963±2.299	10.833±2.424	0.112
NAG index (unit per gram)	0.643±0.172	0.408±0.116	<0.001*

*significant at p-value <0.05, Data are presented as mean ± SD, NAG: N-acetyl-β-D-glucosaminidase

A substantial association was existed among the treatment duration and the urinary NAG index. Increased duration of treatment with sodium valproate is associated with increased urinary NAG index. (r=0.309, P=0.029). Figure 1

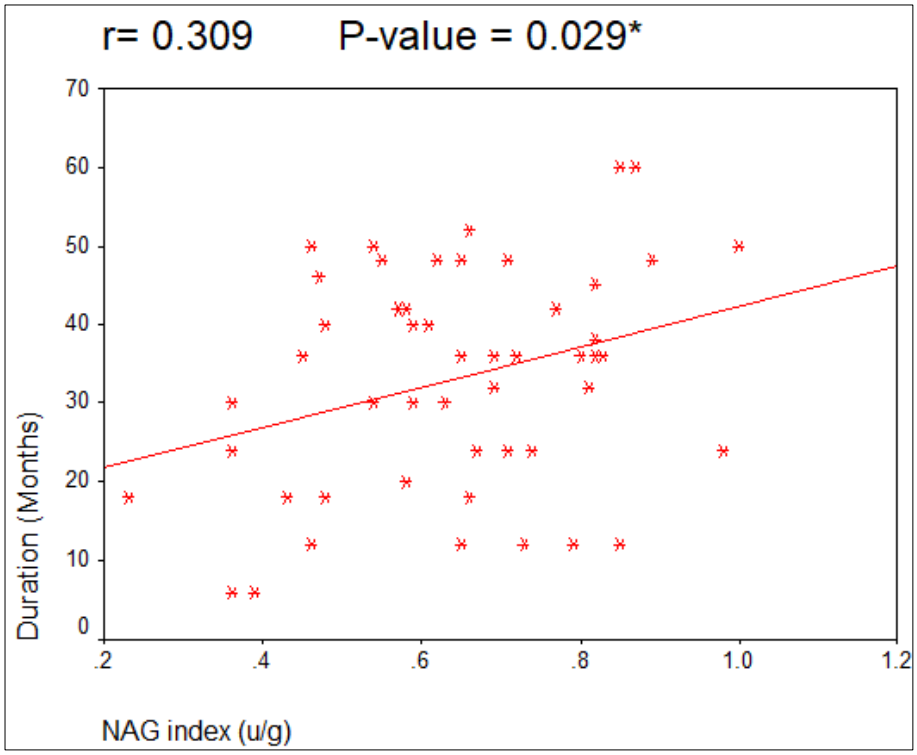

Fig 1: Correlation between the mean urinary NAG index and the duration of treatment with sodium valproate

A substantial association was existed among the mean serum valproate level and urinary NAG index. Increase serum level of sodium valproate was not associated with increase urinary NAG excretion. (r= 0.092, P=0.524). Figure 2

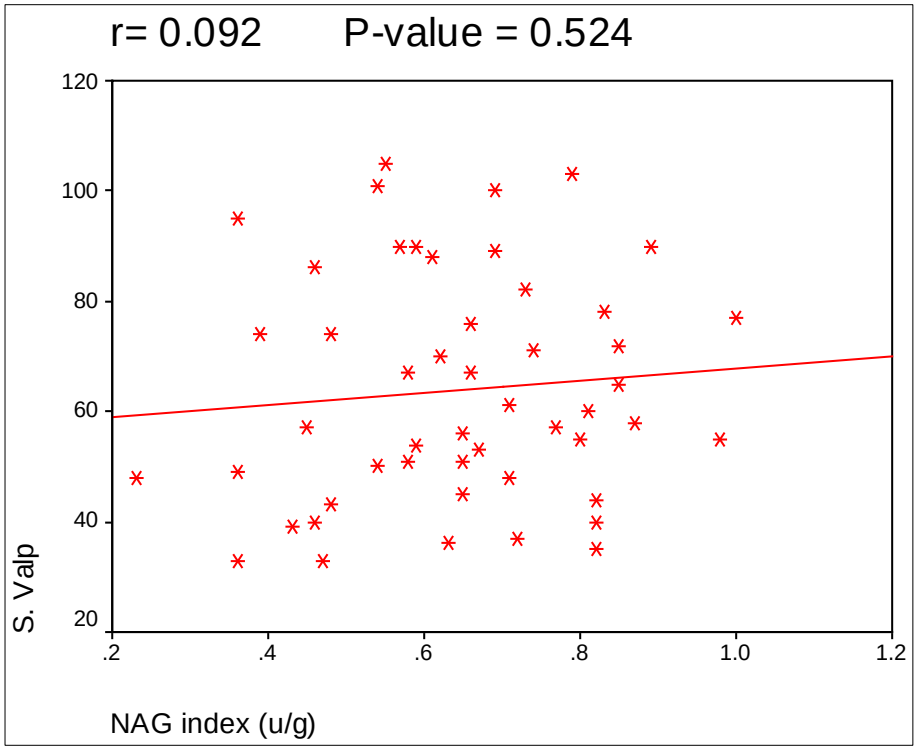

Fig 2: Correlation between the mean urinary NAG index and the mean serum sodium valproate levels in children with epilepsy

Discussion

Because of the broad range of antiepileptic action it exhibits, valproic acid has become one of the most well-known and often given AEDs [18]. It became the most frequently used antiepileptic medication in the world after receiving antiepileptic medicinal approval in France in 1967 [18].

The result of the present study has demonstrated that there was substantial increase in urinary NAG excretion in epileptic children who received sodium valproate treatment. A substantial association was existed among the duration of sodium valproate intake and the mean urinary NAG index. In addition, no substantial association was existed among serum valproate level and urinary NAG index. Respect to the age, sex, serum SGOT, SGPT, urea, creatinine and creatinine clearance, no substantially statistical variation was existed among the children with epilepsy and the control children. Increase the urinary NAG excretion indicates renal tubular insult. Approximately sixty percentile (60%) of patient in this study affected, although creatinine level did not rise.

These results agree with the results obtained by Knights MJ *et al*. [19] who had studied the mean urinary NAG and index of urinary NAG in fifteen (15) children with epilepsy treated by sodium valproate for more than 6 months. The result of the present study showed that a substantially statistical rises in the urinary NAG and urinary NAG index was existed in epileptic children who received sodium valproate treatment than the control children.

However, the findings of this investigation were in conflict with those of Korinthenberg R *et al*. [20] who had studied urinary excretion of NAG in fourteen (14) children who suffered from epileptic seizures and treated with sodium valproate. This study revealed that, at time of diagnosis and before starting valproate therapy, urinary NAG was already increased. Then after 3- 4 months from initiation of the treatment, no further rise was existed in excretion of urinary NAG. It was believed that increase urinary NAG excretion occurred Because of an unidentified physiological component of a medical condition, not an adverse effect of treatment. Additionally, the findings of the present study agree with those of Unay B *et al*. [21] who had studied the urinary NAG activity in forty six (46) children with epilepsy treated with valproate monotherapy with the mean serum concentration of valproate was (68.7 + /- 17.44 microg/mL) as regards to urinary NAG and urinary NAG index but as regard to association across NAG index and serum concentration of sodium valproate there were contradictory.

The danger of acute kidney damage is growing with the expanding employment of therapeutic drugs and diagnostic procedures which are extremely nephrotoxic like sodium valproate [22].

Nevertheless, it is still unclear how VPA causes damage to the kidneys. According to the recent studies, the kidney may be experiencing increased oxidative stress and mitochondrial dysfunction which are suggested as a key risk factor for kidney tissue destruction and organ failure [23, 24].

Valproic acid also causes carnitine deficiencies inflammation, and fibrosis in mouse renal tissue, according to clinical and experimental studies, as well as *in vitro* research [25].

With the informative benefit of being able to distinguish between different types of kidney damage, renal biomarkers aid in an early intervention decision. Nevertheless, the available biomarkers as blood urea nitrogen and creatinine are not sensitive enough to detect harm at an early enough stage to help with the acute prevention of nephrotoxic causes [26]. Therefore, novel biomarkers are being investigated in a variety of patient groups in an effort to discover a marker for acute and chronic renal damage that is more precise, sensitive, and early than creatinine, which has been utilized for decades [27].

NAG is one of these novel biomarkers and the proximal tubule's epithelial cells, which have an unusually high concentration of Lysosomes, are the primary source of urinary NAG [28].

When the glomerular membrane remains intact, NAG cannot enter the glomerular filtrate due to its large molecular weight. Under normal conditions, the low amount of NAG in the urine demonstrates leaking as a result of the epithelial cells' exocytosis and pinocytosis activities.

Higher metabolic activity brought on by raised glomerular filtration and the corresponding increase in reabsorption, higher excretion of exogenous substances (such as drugs and their related metabolites), or cells destruction in the proximal tubule (Due to toxic harm or infections), can all result in increased urinary NAG [29].

So it is always necessary to consider a rise in NAG excretion in the urine as a marker of proximal cell malfunction. And urinary NAG tests could have substantial promise for examining and diagnosing early renal tubular impairment observed in children with epilepsy received sodium valproate treatment [30].

The NAG may be also utilized to monitor as well as detect tubular dysfunction brought on by valproate. As more and more instances of valproate-induced Fanconi syndrome are being documented, this surveillance is becoming more and more important [31].

Conclusion

Sodium valproate has significant time-related adverse effect on functions of kidney. In renal tissues, it causes histopathological alterations and oxidative stress causing tubulointerstitial nephritis. Also, the capacity of the renal tubules for reabsorbing electrolytes, protein, glucose, and urea is being lost in cases of Fanconi syndrome produced by valproate, which is becoming more prevalent. Increased urinary NAG and urinary NAG index in epileptic children received sodium valproate treatment is an early indicator of renal impairment. There is substantial potential association among urinary NAG and treatment duration, The increase in urinary NAG excretion becomes greater as the duration increases.

Financial support and sponsorship: Nil

Conflict of Interest: Nil

References

1. Sarma AK, Khandker N, Kurczewski L, Brophy GM. Medical management of epileptic seizures: challenges and solutions. Neuropsychiatr Dis Treat. 2016;12:467-85.
2. Aaberg KM, Gunnes N, Bakken IJ, Lund Søraas C, Berntsen A, Magnus P, *et al*. Incidence and Prevalence of Childhood Epilepsy: A Nationwide Cohort Study. Pediatrics; c2017, 139.
3. Brodie MJ, Kwan P. Staged approach to epilepsy management. Neurology. 2002;58:S2-8.
4. Goldenberg MM. Overview of drugs used for epilepsy and seizures: Etiology, diagnosis, and treatment. P t. 2010;35:392-415.
5. Löscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs. 2002;16:669-94.
6. Olsen KB, Taubøll E, Gjerstad L. Valproate is an effective, well-tolerated drug for treatment of status epilepticus/serial attacks in adults. Acta Neurol Scand Suppl. 2007;187:51-4.
7. Kwan SY. The role of intravenous valproate in convulsive status epilepticus in the future. Acta Neurol Taiwan. 2010;19:78-81.
8. Balagura G, Iapadre G, Verrotti A, Striano P. Moving beyond sodium valproate: choosing the right anti-epileptic drug in children. Expert Opin Pharmacother. 2019;20:1449-56.
9. Hamed SA. The effect of antiepileptic drugs on the kidney function and structure. Expert Rev Clin Pharmacol. 2017;10:993-1006.
10. Zaki EL, Springate JE. Renal injury from valproic acid: case report and literature review. Pediatr Neurol. 2002;27:318-9.
11. Knights MJ, Finlay E. The effects of sodium valproate on the renal function of children with epilepsy. Pediatric nephrology (Berlin, Germany). 2014;29:1131-8.
12. Skalova S, Chladek J. Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children. Nephrology (Carlton). 2004;9:19-21.
13. Yüksel A, Cengiz M, Seven M, Cengiz S, Cenani A. N-acetyl-beta-glucosaminidase and beta-galactosidase activity in children receiving antiepileptic drugs. Pediatr Neurol. 1999;20:24-6.
14. Bazzi C, Petrini C, Rizza V, Arrigo G, Napodano P, Paparella M, *et al*. Urinary N-acetyl-beta-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. Nephrol Dial Transplant. 2002;17:1890-6.
15. Siddiqui K, Al-Malki B, George TP, Nawaz SS, Rubeaan KA. Urinary N-acetyl-beta-d-glucosaminidase (NAG) with neutrophil gelatinase-associated lipocalin (NGAL) improves the diagnostic value for proximal tubule damage in diabetic kidney disease. 3 Biotech. 2019;9:66.
16. Kalahasthi RB, Rajmohan H, Rajan B, Kumar MK. Urinary N-acetyl-beta-D-glucosaminidase and its isoenzymes A & B in workers exposed to cadmium at cadmium plating. Journal of Occupational Medicine and Toxicology. 2007;2:1-7.
17. Skálová S. The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. ACTA MEDICA-HRADEC KRALOVE-. 2005;48:75.
18. Perucca E. Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. CNS Drugs. 2002;16:695-714.
19. Knights MJ, Finlay E. The effects of sodium valproate on the renal function of children with epilepsy. Pediatr Nephrol. 2014;29:1131-8.
20. Korinthenberg R, Säger J, Zimmerhackl LB. Increased urinary excretion of tubular enzymes and proteins in children with epilepsy. Eur J Paediatr Neurol. 2000;4:263-7.
21. Unay B, Akin R, Sarici SU, Gok F, Kurt I, Gokcay E. Evaluation of renal tubular function in children taking anti-epileptic treatment. Nephrology (Carlton). 2006;11:485-8.
22. Bagshaw SM. Subclinical acute kidney injury: a novel biomarker-defined syndrome. Crit Care Resusc. 2011;13:201-3.
23. Niaudet P, Rotig A. The kidney in mitochondrial cytopathies. Kidney Int. 1997;51:1000-7.

24. Finsterer J. Mitochondriopathies. Eur J Neurol. 2004;11:163-86.
25. Heidari R, Jafari F, Khodaei F, Shirazi Yeganeh B, Niknahad H. Mechanism of valproic acid-induced Fanconi syndrome involves mitochondrial dysfunction and oxidative stress in rat kidney. Nephrology (Carlton). 2018;23:351-61.
26. Al-Naimi MS, Rasheed HA, Hussien NR, Al-Kuraishy HM, Al-Gareeb AI. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharm Technol Res. 2019;10:95-9.
27. Han WK, Bonventre JV. Biologic markers for the early detection of acute kidney injury. Curr Opin Crit Care. 2004;10:476-82.
28. Le Hir M, Dubach UC, Schmidt U. Quantitative distribution of lysosomal hydrolases in the rat nephron. Histochemistry. 1979;63:245-51.
29. Price RG. Urinary enzymes, nephrotoxicity and renal disease. Toxicology. 1982;23:99-134.
30. Price R. Urinary N-acetyl-beta-D-glucosaminidase (NAG) as an indicator of renal disease. Curr probl clin biochem; c1979. p. 150-63.
31. Endo A, Fujita Y, Fuchigami T, Takahashi S, Mugishima H. Fanconi syndrome caused by valproic acid. Pediatr Neurol. 2010;42:287-90.

How to Cite This Article
Abdalla HR, Aziz SAEAAE, Elsrogy HA, Hantash HI. Urinary N-acetyl - βeta -D- glucosaminidase (NAG) in children with epilepsy treated with sodium valproate monotherapy. International Journal of Pediatrics and Neonatology. 2024;6(1):05-10.