INJ INTERNATIONAL NEUROUROLOGY JOURNAL

Influence of UGT1A3 Polymorphism on Sodium Valproate Monotherapy And Clinical Outcome in Pediatric Epileptic Patients: A Cohort study

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Abstract

Objective: The objective of this study was to assess the association between UGT1A3 genetic polymorphism and clinical outcome in terms of efficacy and tolerability in pediatric epileptic patients on sodium valproate monotherapy.

Methodology: This Prospective Cohort study recruited hundred Pediatric epileptic patients in the age group of 2-18 years, receiving sodium valproate as monotherapy at least for 1 month. Blood sample were collected after obtaining consent for genetic polymorphism analysis, to estimate serum drug concentration and biochemical parameters and for platelet estimation. Genomic DNA from whole blood was isolated by Phenol-chloroform extraction and ethanol precipitation method which was then processed for amplification by PCR followed by Sequencing method for mutation analysis. Biochemical investigations were performed by hematology cell counter. Clinical outcome was assessed in terms of efficacy and safety of sodium valproate.

Results: It was observed that majority of wild varieties of A17G and C133T suffered either with GTCS or partial seizures suggesting that the mutation would be beneficial in reducing the risk of seizures. Vice versa holds good for T31C, G81A, T140C and A477G. Patients with mutant varieties were epileptic in these genotypes suggesting that these mutations were dangerous. There was no significant difference in the liver function tests at Basal, 6 months and 1 year as well as between the genotypes. Even though serum creatinine levels were in the biological reference range. There was no significant association between gene polymorphisms and serum VPA concentrations, though VPA levels were significantly different in different intervals.

Conclusion: UGT1A3 gene polymorphism studies may be used before initiating sodium valproate treatment to predict the treatment response. The drug was well tolerated in our study population without much change in hepatic, renal, and pancreatic parameters. Sodium valproate was well-tolerated among pediatric patients with epilepsy and can be used as an effective anti-epileptic drug.

Key words: UGT, gene polymorphism, sodium valproate, clinical outcome

Introduction

Background:

Chronic neurological disorder epilepsy is characterised by uncontrollable seizures that occur often [1]. Statistics show that 40 to 70 people out of every 100,000 people experience epilepsy each year [2]. Paediatric epilepsy affects roughly 22 people per 1000 people [3]. However, according to other research carried out across India, the prevalence of epilepsy is approximately 4–8/1000 people [4,5].

Broad-spectrum antiepileptic medication sodium valproate (VPA) is prescribed as initial treatment for minor and intractable seizures. However, it demonstrates clear individual variability in pharmacokinetics and pharmacodynamics, which implies that even with the same doses of VPA, the serum levels of each patient varies. In order to use doses most effectively during the course of therapy, the serum concentration of VPA should be monitored [6]. The variations in serum concentration might represent functional effects of genetic variables, various illnesses, and individual behaviour [7-9].

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ISSN:2093-4777 | E-ISSN:2093-6931
DOI: 10.5123/inj.2023.4.inj14
Vol. 27 Iss. 4 (2023)

Pharmacogenetics is the study of how genetic variants affect drug metabolism, pharmacological targets, or disease pathways, resulting in changes in how well a medicine works or how many side effects it has [10,11].The concept of "individualized medicine" is evolving and there has been a paradigm shift from the concept of "one drug fits all" to "right drug for the right patient at the right dose and time." Therefore, it is crucial to look at how genetic polymorphisms may affect the pharmacokinetics and pharmacodynamics of antiepileptic medicines.

VPA is extensively metabolised in the liver through cytochrome P450 (CYP)-dependent -, (-ω1)-, and (ω-2)-oxidation, mitochondrial -oxidation, and microsomal glucuronide conjugation [initiated by uridine 5'-diphospho (UDP) glucuronosyltransferase (UGT)]. Less than 5% of it is eliminated in urine unaltered. The well-studied CYP isoforms CYP2C9, CYP2A6, CYP2B6, and perhaps CYP2C19 are involved in the metabolism of valproate.

Four families of UGT genes, including UGT1, UGT2, UGT3, and UGT8, have been found to be involved in the glucuronidation of VPA. Each family comprises of a number of distinct genes that are encoded in distinct complex loci on several chromosomes. It is evident that the glucuronidation of endo- and xenobiotics only involves enzymes from the UGT1 and UGT2 families. Therefore, in comparison to the other UGT families, both families are anticipated to play a significant role in the metabolism of VPA.

The UGT isozymes UGT1A3, UGT1A6, and UGT2B7 all play crucial roles in the synthesis of VPA glucuronides [12,13]. The main UGT isoforms that can glucuronidate valproic acid are UGT1A6, UGT1A9, and UGT2B7, and this route accounts for around 50% of valproate metabolism [14].

Only a few Indian reports on UGT polymorphism are now accessible when it comes to sodium valproate. The steady-state concentration of VPA was found to be significantly influenced by the UGT1A6 552 A>C polymorphism, according to Munisamy et al. [15]. In the North Indian patient group, Jain et al. revealed the incidence of UGT 1A6 polymorphism in children receiving valproate monotherapy. Small sample size, just one UGT isoform was examined, and the incidence of UGT polymorphism in the general population were study limitations. The study's shortcomings prevented it from conclusively proving a relationship between serum valproic acid levels and certain UGT 1A6 polymorphisms[16]. Indian children with epilepsy's genetic variants of UGT1A6 were examined, and their potential impact on the pharmacokinetics of valproate was determined.
To the best of our knowledge, we could only locate a small number of reports regarding UGT1A3 polymorphism.UGT1A3 and plasma levels of valproic acid have been linked, according to Shenghui et al [17].In conclusion, UGT1A3 may be a significant determinant of individual variability in the pharmacokinetics of VPA, and it may be necessary to increase VPA dose among its carriers to ensure its therapeutic range, according to Xiao-Man et al's study of the influence of UGT 1A3 polymorphism in the Chinese epileptic population[18].Another Chinese study by Xiongrong et al. found that the polymorphisms of UGT1A3 A17G and UGT1A3 C133T have an impact on the levels of valproic acid[19]. We therefore want to look at how the UGT1A3 polymorphism affects the steady state valproic acid levels in paediatric epileptics.

The significant variation in dosage across individuals may be a functional effect of genetic variations in genes encoding drug-metabolizing enzymes. The idea of "individualised medicine" is developing, and the idea of "one drug fits all" has given way to the idea of "right drug for the right patient at the right dose and time." Therefore, it is crucial to look into any potential involvement that genetic polymorphisms may have in the metabolism of VPA. To the best of our knowledge, there aren't many studies on the impact of UGT1A3 gene polymorphism on VPA metabolism, particularly in Indian contexts.

Objectives of the Study were to,

1) evaluate the pattern of polymorphism in UGT1A3 in patients on valproate monotherapy of pediatric epileptic patients and find its association with plasma concentration of valproate
2) find the association between the genetic polymorphism of UGT1A3 with clinical effectiveness and adverse effect profile of sodium valproate.
3) assess the adverse effects of Sodium valproate by analysing LFT, haematological, clinical parameters and correlate it with genetic polymorphisms

Methodology

Study setting: The study was conducted at Central Research Laboratory of K S Hegde Medical Academy and Department of Pediatrics, Justice K S Hegde Charitable Hospital of Nitte (Deemed to be University), Mangalore, Karnataka, India

Type of study: Prospective Cohort
Study subjects:

Inclusion criteria: Hundred Pediatric epileptic patients in the age group of 2-18 years, diagnosed based on their seizure history as well as electroencephalogram tests, receiving sodium valproate as monotherapy at least for 1 month were recruited into the study.

Exclusion criteria: Children receiving other antiepileptic drugs, drugs which may interfere with valproate metabolism or herbal or alternative medicine, patients with hepatic, renal abnormalities based on clinical and laboratory investigations.

Sample Collection and processing: Patients were informed to skip the previous dose (night) and blood sample was collected on next morning for trough level concentration estimation. 5ml of whole blood sample was collected with aseptic precaution after 30 days treatment of sodium valproate. Sample was separated into 3 parts; 2ml of EDTA whole blood stored at -80º C for genetic polymorphism analysis, 2 ml of whole blood in plain vial centrifuged to obtain serum and then stored at -80º C to estimate serum drug concentration and biochemical parameters and 1ml of EDTA whole blood for platelet estimation.

Genotyping:
Genomic DNA from whole blood was isolated by Phenol-chloroform extraction and ethanol precipitation method as follows ;

DNA isolation (Miller's et al. method): Blood sample was centrifuged with RBC lysing solution (NH_4Cl, $KHCO_3$, Na_2. EDTA). The residual RBC lysate was suspended with a cell lysing solution [50mM Tris HCl, 50mM EDTA, 10mM NaCl, 1% Sodium dodecylsulphate (SDS)] and the cell lysates was digested overnight followed by the addition of protein precipitating solution. After centrifugation (3000 RPM,10 mins), the supernatant was collected in the 2% isopropanol and centrifuged again. The pellet which consists of DNA was washed with 70% ethanol and the DNA was allowed to precipitate. The precipitated DNA was transferred to the vials containing 50 µl of TE buffer (pH 7.5).

The quantity of the DNA isolated was determined using a nanodrop spectrophotometer (Eppendorf) at 260nm. OD 260/280 ratio for considering the purity of DNA extracted was between 1.8-2 [20]. Quantified DNA was sealed and stored at -20°C until further analysis.

The UGT1A3 gene of 519 base pair length was amplified by the Polymerase Chain Reaction (PCR) method. Amplification was performed in MJ-Mini Thermal cycler (Bio-Rad, Tokyo, Japan). Primer used was:
Forward: 5'-CGTGTCTTCTGCTGAGATGG-3'
Reverse: 5'-GGAATCGACAGGTACTTAGCC-3'
PCR was conducted with an initial denaturation enzyme activation step at 95°C for 5 minutes, amplification step for 35 cycles at 95°C for 30 seconds, an annealing temperature of 62°C for 30 seconds, treatment at 72°C for 30 seconds and a final extension step at 72°C for 5 minutes. Amplified product of DNA samples was confirmed on a 2% agarose gel with ethidium bromide. For confirmed observation of mutation in the gene sequence, **sequencing technique** was carried out which provides the exact mutation location in the sequence pattern.

Steady-state trough serum concentration of sodium valproate was determined by High-Performance Liquid Chromatography (HPLC) analysis (Agillent Technologies, 1260 Infinity). Trough serum concentrations of sodium valproate was standardized at 20, 40, 80, 120, 160, 200 µg/ml. Nonanoic acid was used as internal standard (I.S.) (2mg/ml). Reverse phase column of the dimensions C18 (250 × 4.6 mm internal diameter) maintained at a temperature of 35 °C were used. Mobile phase consisted of acetonitrile and 29mM phosphate buffer (pH 3.5) 45: 55 v/v was used at a flow rate of 1.2ml/min. Wavelength used was 220 nm during the process. The method was linear over a concentration range of 20 - 200 µg/ml for valproic acid. The total run

time was set for 10 minutes. Retention time for Sodium Valproate was 4.5 min and internal standard nonanoic acid was 8.3 min.

Biochemical investigations like albumin, total protein, AST, ALT, ALP, total bilirubin, direct bilirubin, serum amylase, creatinine and urea was estimated using fully automated clinical chemistry analyzer (COBAS C311), platelet count was performed on hematology cell counter.

Clinical outcome was assessed in terms of efficacy and safety of sodium valproate. The efficacy of the drug was measured in terms of treatment responders or non-responders. Responder to sodium valproate was defined as patients with no relapse or <2 episodes of seizure attack in 1 year of follow up and is well tolerated with sodium valproate monotherapy without any adverse effect seen. Non-responders were those with two or more seizure attack episodes in 1 year of follow-up, or any adverse drug effect is seen after therapy initiation. Safety was assessed by comparing the biochemical parameters between basal, six-month, and 1-year interval. Parents were asked to bring their children hospital if any untoward response (loss of appetite, nausea, vomiting, discoloration of eye, pain abdomen, rashes) to the treatment at any point of time [21].

Ethical Issue: Central Ethics Committee (NU) approval was obtained prior to the study. (Approval letter No: NU/CEC/2019/0223). Written Informed Consent was taken from parents or first-degree relatives of patients.

Statistical analysis:
The collected information was summarized by using frequency, the percentage for qualitative data, and mean ± SD for quantitative data. Hardy-Weinberg equilibrium (HWE) was calculated by using an online "wpcalc" calculator. Gene polymorphism and Sodium valproate levels were compared by using One Way ANOVA and student "t' test. Association between genetic polymorphism and clinical outcome was assessed by the Chi-Square test. The "P" value.

Results

One hundred twenty-two patients were screened, out of which a hundred patients fulfilled inclusion criteria and enrolled in the study after signing informed consent. Hundred patient samples were collected during the time of enrolment, 81 patients during six months follow-up investigations were performed, and one-year follow-up was carried out for 72 patients. Eleven patients shifted from sodium valproate treatment to other anti-seizure drug therapy, and in eight patients, another anti-seizure drug was added along with sodium valproate because of recurrent seizure attacks. One patient died because of complications of congenital heart disease. We could not collect samples of eight patients at six months and one year; however, they were seizure-free during this period (Figure 1).

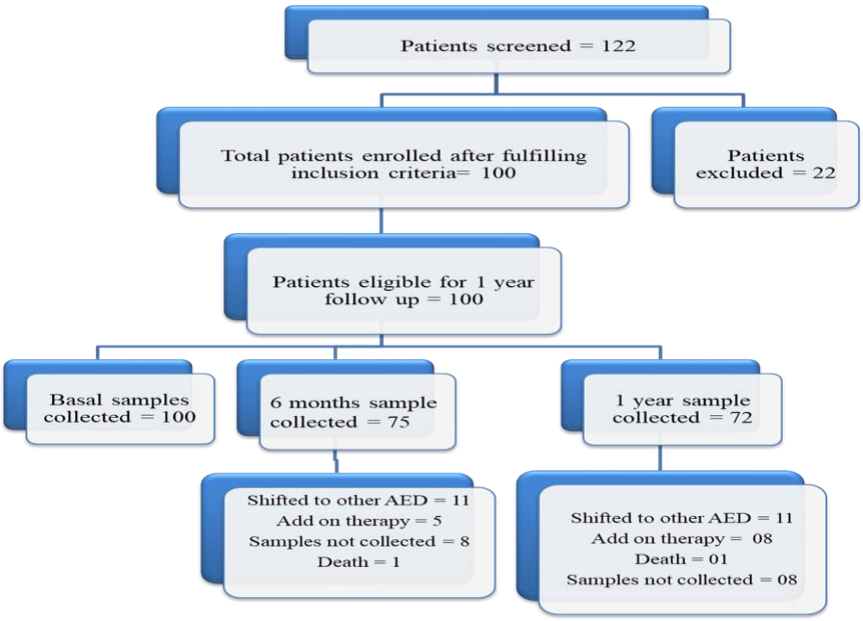

Figure 1: STROBE Flow chart

The mean age of study participants was 8.5 ± 4.3 years (2.2 years — 17.3years), and the average BMI was 16.5 ± 4.3 (7.81 – 32.84) during enrolment. Fifty-seven patients were males, and forty-three patients were females. Patients were categorized into 3 groups based on age (i.e., 2-6 yrs, 6-12yrs and 12-18 yrs). There was a statistically significant difference observed in BMI between different age groups. However, the BMI was in the normal range in different age group patients. (Table 1)

Table 1: Demographic data of patients in different age groups at baseline

Characteristics	**2-6 years (N=25)**	**6-12 years (N=60)**	**12-18 years (N=15)**	**P-value**
Gender- Male:	17	32	08	
Female:	08	28	07	
Seizure type- GTCS:	17	55	11	
CPS:	08	05	04	
BMI (Mean ± SD)	14.08 ± 3.5	16.08 ± 3.8	21.04 ± 4.4	**0.000***
Sodium Valproate Concentration (µg/ml) **(Mean ± SD)**	95.7 ± 43.6	104.2 ± 28.5	112.3 ± 23.3	0.315

One-way ANOVA followed by Bonferroni's post hoc test.
*P-value <0.05 considered statistically significant

UGT1A3 A17G on sequencing showed that wild was 99% and mutant allele was only 1%. 100%of the patients showed mutation in T31C genotype. Sequencing of G81A showed that 14% were wild type and 86% were mutant. There was no mutation at all for C133T genotype. T140C analysis revealed 6% of the patients had wild type and 94% mutant variety. A477G showed 27% wild variety and 73% mutant type (table 2). Sequencing results are depicted in 3A-3E.

Table 2: UGT1A3Sequencing results of study participants

UGT1A3											
A17G		***T31C***		***G81A***		***C133T***		***T140C***		***A477G***	
Wild A	*99*	*Wild T*	*0*	*Wild G*	*14*	*Wild C*	*100*	*Wild T*	*6*	*Wild A*	*27*
Mutant G	*01*	*Mutant C*	*100*	*Mutant A*	*86*	*Mutant T*	*0*	*Mutant C*	*94*	*Mutant G*	*73*

Patients with wild alleles of A17G showed a decline in VPA concentrations, (11.3% at 6 months 20.02% at 1 year) (Table 3). Patients with T31C also followed the similar pattern but alleles were of mutant variety. Both wild and mutant varieties of G81A exhibited decline in the VPA concentration at 6 months and 1 year.
Patients with other genotypes, C133T, T140C and A477G also had a fall in VPA concentration (both wild and mutant) in the similar pattern. However, the p values were not calculated as some of the groups had minimum number of values needed for statistical analysis.

Table 3: Steady state Sodium Valproate concentration in different genotype

Genotype	**Basal (n=100) (µg/ml)**	**6 months (n=81) (µg/ml)**	**1 year (n=72) (µg/ml)**

	WILD	MUTANT	WILD	MUTANT	WILD	MUTANT
UGT1A3 (A17G)	103.3±34.28	104.0	92.53±42.09	63.00	77.16±34.70	142.0
UGT1A3 (T31C)	-	103.3±34.10	-	92.15±41.95	-	78.03±35.27
UGT1A3 (G81A)	99.36±29.98	104.0±34.84	86.50±47.21	93.20±41.21	77.17±41.69	78.19±34.29
UGT1A3 (C133T)	103.3±34.10	-	92.15±41.95	-	78.03±35.27	-
UGT1A3 (T140C)	103.5±38.13	77.25±45.75	98.75±54.76	91.78±41.58	77.25±45.75	78.07±35.00
UGT1A3 (A477G)	98.89±31.87	105.0±34.96	93.74±41.87	91.50±42.35	82.95±31.21	75.98±36.91

It can be observed in table 4 that majority of wild varieties of A17G and C133T suffered either with GTCS or partial seizures suggesting that the mutation would be beneficial in reducing the risk of seizures. Vice versa holds good for T31C, G81A, T140C and A477G. Patients with mutant varieties were epileptic in these genotypes suggesting that these mutations were dangerous.

Table 4: Pattern of UGT1A3 gene polymorphisms with seizure types

GENOTYPE	GTCS(N=83)		PARTIAL SEIZURE(N=17)	
	WILD	MUTANT	WILD	MUTANT
UGT1A3 (A17G)	82	1	17	-
UGT1A3 (T31C)	-	83	-	17
UGT1A3 (G81A)	12	71	2	15
UGT1A3 (C133T)	83	-	17	-
UGT1A3 (T140C)	5	78	1	16
UGT1A3 (A477G)	23	60	4	13

Albumin, total protein, SGOT, alkaline phosphatase, bilirubin total, and serum creatinine values showed a significant difference in patients of different age groups during enrolment. (Table 5)

Table 5: Comparison of biochemical and hematological parameters in differentage groups at baseline.

Characteristics	2-6 years (Mean ± SD)(N=25)	6-12 years (Mean ± SD)(N=60)	12-18 years (Mean ± SD)(N=15)	P-value
Albumin (g/dL)	4.31 ± 0.31	4.47 ± 0.37	4.62 ± 0.20	**0.023***
Total protein (g/dL)	6.98 ± 0.50	7.45 ± 0.68	7.56 ± 0.29	**0.003***
SGOT (IU/L)	38.5 ± 17.1	30.5 ± 13.6	21.8 ± 5.7	**0.047***
SGPT (IU/L)	14.2 ± 5.01	13.7 ± 4.88	13.4 ± 5.26	0.951
Alkaline phosphatase (IU/L)	208.3 ± 72.6	198.4 ± 51.3	141.7 ± 64.2	**0.010***
Direct bilirubin (mg/dL)	0.11 ± 0.03	0.11 ± 0.04	0.10 ± 0.03	0.524
Total bilirubin (mg/dL)	0.22 ± 0.09	0.30 ± 0.13	0.41 ± 0.19	**0.020***
Blood urea (mg/dL)	22.16 ± 7.9	20.6 ± 7.13	20.7 ± 8.5	0.696
Serum creatinine (mg/dL)	0.33 ± 0.14	0.50 ± 0.19	0.68 ± 0.19	**0.000***
Amylase (IU/L)	76.28 ± 30.2	89.13 ± 41.3	83.8 ± 40.2	0.528
Platelets (cells/mm^3)	305760 ± 75870	271230 ± 59830	288670 ± 55967	0.074

One-way ANOVA followed by Bonferroni's post hoc test.
*P-value <0.05 considered statistically significant

Comparison of drug concentration, biochemical and hematological parameters at different time intervals were depicted in table 6. VPA concentrations significantly decreased over the 1-year period. Serum creatinine was in the biological reference range even though significant difference was there when basal,6 months and 1-year values.

Table 6: Drug concentration, Biochemical and Hematological parameters at different time intervals

Characteristics	Basal **(n=100)**	6 months(n=81)	1 year (n=72)	P value
	Mean± SD	**Mean± SD**	**Mean± SD**	
BMI	16.51±4.30	16.69±3.71	17.37±3.60	0.354
Sodium Valproate Concentration (µg/ml)	103±34.10	92.15±41.95	78.03±35.27	<0.0001**
Albumin (g/dl)	4.44±0.38	4.43±0.34	4.43±0.35	0.99
Total Protein (g/dl)	7.34±0.64	7.49±0.615	7.49±0.71	0.241
SGOT (IU/L)	29.41±16.71	27.25±8.30	28.87±8.36	0.512
SGPT (IU/L)	13.55±8.173	12.63±4.88	12.40±5.26	0.464

Alkaline Phosphate (IU/L)	192.7±72.75	204±79.90	206±80.08	0.461
Direct Bilirubin (mg/dl)	0.10±0.11	0.108±0.054	0.113±0.08	0.860
Total Bilirubin (mg/dl)	0.29±0.11	0.297±0.16	0.309±0.186	0.917
Blood Urea (mg/dl)	20.94±7.55	19.23±7.28	19.85±6.65	0.284
Serum Creatinine (mg/dl)	0.48±0.21	0.397±0.137	0.390±0.124	0.0002**
Amylase (IU/L)	85.19±43.04	79.71±32.83	77.59±32.80	0.383
Platelet (cells/cubic mm)	281960±65080	27733±42979	295845±30500	0.715

*p highly significant

80% of the patients with wild alleles of A17G were responders, implying that even though they were at risk of epilepsy, enzyme UGT1A3 was effectively metabolizing VPA in them (Table 7) whereas patients with T31C, G81A, T140C and A477G mutation was beneficial in maintaining VPA concentration in therapeutic range and have majority of the patients were responders. However, table 8 shows that there was no significant association between the gene polymorphism and clinical outcome in pediatric epileptics.

Table 7: Pattern of UGT1A3 gene polymorphisms with clinical outcome.

Genotype	Clinical response (Efficacy)			
	WILD		MUTANT	
	Responders (%)	Non- responders (%)	Responders (%)	Non- responders (%)
A17G	80	19	-	1
T31C	-	-	80	20
G81A	10	4	70	16
C133T	80	20	-	-
T140C	5	1	75	19
A477G	23	4	57	16

Table 8: Association of UGT1A3 gene polymorphisms with clinical outcome.

UGT1A3 Genotype	Polymorphism pattern	Polymorphism in percentage	Clinical outcome		P value
			Responders (n=80)	Non responders (n=20)	
UGT1A3 (A17G)	WILD	99	80	0	-
	MUTANT	1	19	1	
UGT1A3 (T31C)	WILD	0	0	0	-
	MUTANT	100	80	20	
UGT1A3 (G81A)	WILD	14	10	4	0.38
	MUTANT	86	70	16	
UGT1A3 (C133T)	WILD	100	80	20	-
	MUTANT	0	0	0	
UGT1A3 (T140C)	WILD	6	5	1	0.83
	MUTANT	94	75	19	
UGT1A3 (A477G)	WILD	27	23	4	0.43
	MUTANT	73	57	16	

Chi- square test, P<0.05 considered significant

There was no significant difference in the liver function tests at Basal, 6 months and 1 year as well as between the genotypes. Even though serum creatinine levels were in the biological reference range. There was a significant decline in the levels at 6 months and 1 year for patients with genotypes A17G, T31C, G81A, C133T. Creatinine levels did not differ significantly in patients with T140C and A477G at different intervals. Blood urea, serum amylase and platelet count also insignificantly differ in all the genotypes (Table no 10 A- 10F)

Table 9: Association of UGT1A3 gene polymorphisms with seizure types

UGT1A3 Genotype	Polymorphism pattern	Polymorphism in percentage	Seizure type		P value
			GTCS (n=83)	Focal seizures (n=17)	
UGT1A3 (A17G)	WILD	99	82	17	-
	MUTANT	1	1	0	
UGT1A3 (T31C)	WILD	0	0	0	-
	MUTANT	100	83	17	
UGT1A3 (G81A)	WILD	14	12	2	0.77
	MUTANT	86	71	15	
UGT1A3 (C133T)	WILD	100	83	17	-
	MUTANT	0	0	0	
UGT1A3 (T140C)	WILD	6	5	1	0.98
	MUTANT	94	78	16	
UGT1A3 (A477G)	WILD	27	23	4	0.73
	MUTANT	73	60	13	

Table 10A: Comparison of biochemical parameters in genotype at different intervals

		WILD Mean± SD	P value	MUTANT Mean± SD	P value	WILD	P value	MUTANT Mean± SD	P value
Albumin (g/dL)	Basal	4.439±0.384	0.9808	4.70	-	-	-	4.442±0.3833	0.99
	6 months	4.439±0.348		4.5				4.439±0.34	
	1 year	4.43±0.35		4.8				4.434±0.354	
Total Protein (g/dL)	Basal	7.351±0.64	0.24	7.2	-	-	-	7.34±0.64	0.24
	6 months	7.49±0.617		7.1				7.49±0.615	
	1 year	7.49±0.72		7.7				7.49±0.711	
SGOT(U/L)	Basal	29.47±16.78	0.53	23	-	-	-	29.41±16.71	0.51
	6 months	27.35±8.31		20				27.25±8.30	
	1 year	28.98±8.37		21				28.87±8.37	
SGPT(U/L)	Basal	13.63±8.17	0.463	6	-	-	-	13.55±8.17	0.46
	6 months	12.70±4.87		7				12.63±4.88	
	1 year	12.46±5.27		8				12.40±5.26	
ALP (IU/L)	Basal	192.7±73.12	0.423	199	-	-	-	192.7±72.75	0.46
	6 months	204.5±80.31		165				204±79.90	
	1 year	207±80.28		142				206.2±80.08	

ANOVA test, P<0.05 considered significant

Table 10B: Comparison of biochemical parameters in genotype at different intervals

Parameters		A17G				T31C			
		WILD Median (IQR)	P value	MUTANT	P value	WILD	P value	MUTANT Median (IQR)	P value
Direct bilirubin (mg/dl)	Basal	0.090(0.05-0.12)	0.87	0.06	-	-	-	0.09(0.05-0.12)	0.86
	6 months	0.10(0.07-0.14)		0.07				0.10(0.07-0.14)	
	1 year	0.10(0.07-0.13)		0.1				0.10(0.07-0.13)	
Total bilirubin (mg/dl)	Basal	0.26(0.17-0.36)	0.91	0.17	-	-	-	0.255(0.17-0.36)	0.92
	6 months	0.26(0.18-0.37)		0.20				0.26(0.18-0.37)	
	year	0.25(0.20-0.37)		0.20				0.25(0.20-0.37)	
Urea (mg/dl)	Basal	20(15-26)	0.24	28.0	-	-	-	20(15.08-26.38)	0.28
	6 months	19.10(13.60-23.00)		32.90				19.20(13.60-23.00)	

	1 year	20.40(15.00-24.45)		27.70				20.50(15-24.60)	
Creatinine (mg/dl)	Basal	0.44(0.33-0.60)	0.0003***	0.58	-	-	-	0.44(0.33-0.59)	0.0002***
	6 months	0.38(0.33-0.46)		0.33				0.38(0.33-0.46)	
	1 year	0.37(0.32-0.46)		0.44				0.38(0.32-0.46)	
Amylase (U/L)	Basal	78(56-102)	0.387	96.00	-	-	-	78(56.00-102.00)	0.383
	6 months	75.50(51.75-101)		128.0				77(52-101)	
	1 year	74(51.75-96.50)		67.00				74(52-96)	
Platelet (Lakhs)	Basal	288000(2382500-310500)	0.050	268000	-	-	-	288000(219000-320750)	0.07
	6 months	294500(238250-310500)		283000				294000(239000-310000)	
	1 year	303000(285500-314000)		212000				302000(284000-314000)	

ANOVA test, P<0.05 considered significant

Table 10C: Comparison of biochemical parameters in genotype at different intervals

Parameters		G81A				C133T			
		WILD Mean± SD	P value	MUTANT Mean± SD	P value	WILD Mean± SD	P value	MUTANT	P value
Albumin (g/dL)	Basal	4.44±0.431		4.437±0.378		4.44±0.383	0.99	-	-
	6 months	4.48±0.489	0.784	4.431±0.323	0.94	4.439±0.346			
	1 year	4.573±0.38		4.417±0.348		4.43±0.35			
Total Protein (g/dL)	Basal	7.25±0.44		7.36±0.67		7.34±0.64		-	-
	6 months	7.49±0.82	0.29	7.49±0.57	0.45	7.49±0.615			
	1 year	7.70±0.82		7.45±0.688		7.49±0.711	0.24		
SGOT (U/L)	Basal	26.43±8.16		29.90±17.70		29.41±16.71		-	-
	6 months	25.17±8.08	0.89	27.64±8.344	0.57	27.25±8.30			
	1 year	26.59±8.07		29.29±8.42		28.87±8.37	0.51		
SGPT (U/L)	Basal	13.43±6.50		13.57±8.45		13.55±8.17		-	-
	6 months	11.42±4.50	0.27	12.86±4.95	0.74	12.63±4.88	0.46		

	1 year	10.15±3.01		12.81±5.50		12.40±5.26			
ALP (IU/L)	Basal	202±42.91		74.89		192.7±72.75		-	-
	6 months	195±51.62	0.299	204.8±82.12		204±79.90			
	1 year	234.6±28.87		204.1±82.39	0.78	206.2±80.08	0.46		

ANOVA test, P<0.05 considered significant

Table 10D: Comparison of biochemical parameters in genotype at different intervals

Parameters		G81A				C133T			
		WILD Median (IQR)	P value	MUTANT Median (IQR)	P value	WILD Median (IQR)	P value	MUTANT	P value
Direct bilirubin	Basal	0.07(0.03-0.13)		0.09(0.06-0.12)		0.09(0.05-0.12)		-	-
(mg/dl)	6 months	0.11(0.07-0.16)	0.24	0.10(0.06-0.13)	0.81	0.10(0.07-0.14)	0.86		
	1 year	0.10(0.06-0.11)		0.10(0.08-0.13)		0.10(0.07-0.13)			
Total bilirubin	Basal	0.22(0.15-0.39)		0.26(0.17-0.35)	0.72	0.255(0.17-0.36)		-	-
(mg/dl)	6 months	0.31(0.22-0.42)	0.40	0.24(0.17-0.37)		0.26(0.18-0.37)	0.92		
	1 year	0.28(0.20-0.37)		0.25(0.19-0.37)		0.25(0.20-0.37)			
Urea (mg/dl)	Basal	20.25(13.45-28.00)		19.75(15.45-26.13)		20(15.08-26.38)		-	-
	6 months	22.15(14.15-24)	0.87	19.00(13.60-22.60)	0.28	19.20(13.60-23.00)			
	1 year	20.60(11.10-24.80)		19.75(15.10-24.55)		20.50(15-24.60)	0.28		
Creatinine (mg/dl)	Basal	0.53(0.38-0.64)		0.44(0.31-0.58)		0.44(0.33-0.59)		-	-
	6 months	0.38(0.32-0.47)	0.058	0.38(0.34-0.45)	0.0015**	0.38(0.33-0.46)			
	1 year	0.39(0.37-0.46)		0.37(0.31-0.45)		0.38(0.32-0.46)	0.0002***		
Amylase (U/L)	Basal	74(53.75-97.50)	0.75	78.50(56.00-104.5)	0.43	78(56.00-102.00)	0.383	-	-

	6 months	69.50(46.75-89.00)		79(53-101)		77(52-101)				
	1 year	67(58-118)		74(51.25-95.0)		74(52-96)				
Platelet (Lakhs)	Basal	297000(268000-313000)		287000(218000-322500)		288000(219000-320750)		0.07	-	-
			0.96		0.066					
	6 months	295000(283250-310000)		294000(236000-310000)		294000(239000-310000)				
	1 year	302000(281000-314000)		305000(286000-313500)		302000(284000-314000)				

ANOVA test, P<0.05 considered significant

Table 10E: Comparison of biochemical parameters in genotype at different intervals

ANOVA test, P<0.05 considered significant

Parameters		T140C				A477G			
		WILD Mean± SD	P value	MUTANT Mean± SD	P value	WILD Mean± SD	P value	MUTANT Mean± SD	P value
Albumin (g/dL)	Basal	4.417±0.142		4.44±0.39		4.44±0.383	0.99	4.45±0.35	
	6 months	4.37±0.26	0.61	4.44±0.35	0.944	4.439±0.346		4.37±0.31	
	1 year	4.58±0.49		4.425±0.35		4.43±0.35		4.39±0.34	0.45
Total Protein (g/dL)	Basal	7.66±0.41		7.32±0.65		7.34±0.64		7.36±0.688	
	6 months	7.57±0.83	0.936	7.48±0.60	0.210	7.49±0.615		7.40±0.61	
	1 year	7.77±1.10		7.47±0.69		7.49±0.711	0.24	7.48±0.76	0.67
SGOT (U/L)	Basal	24.5 ±5.75		29.72±17.14		29.41±16.71		30.47±18.64	
	6 months	27.25±8.34	0.771	27.25±8.35	0.45	27.25±8.30		27.73±9.10	
	1 year	25.38±2.28		29.08±8.55		28.87±8.37	0.51	29.33±8.88	0.55
SGPT (U/L)	Basal	14.50±4.27		13.49±8.3		13.55±8.17		14.19±8.7	
	6 months	12.25±4.03		12.65±4.95		12.63±4.88	0.46	12.53±5.06	
	1 year	14.23±3.6	0.67	12.29±5.38	0.49	12.40±5.26		12.24±5.46	0.231
ALP (IU/L)	Basal	177.7±58.25		193.7±73.73		185.2±66.65		195.1±74.8	
	6 months	196.2±12.93	0.66	204.5±82.65		216.2±99.39		198.9±70.74	
	1 year	197.6±31.61		206.9±82.70	0.52	224.6±100.7	0.26	198.5±69.42	0.946

Table 10F: Comparison of biochemical parameters in genotype at different intervals

Parameters		T140C				A477G			
		WILD Median (IQR)	P value	MUTANT Median (IQR)	P value	WILD Median (IQR)	P value	MUTANT Median (IQR)	P value
Direct bilirubin (mg/dl)	Basal	0.08(0.03-0.14)	0.79	0.09(0.05-0.12)	0.90	0.09(0.06-0.12)	0.166	0.08(0.05-0.13)	0.89
	6 months	0.09(0.04-0.15)		0.10(0.07-0.13)		0.11(0.08-0.16)		0.10(0.06-0.13)	
	1 year	0.12(0.08-0.14)		0.10(0.07-0.12)		0.10(0.08-0.11)		0.10(0.06-0.13)	
Total bilirubin (mg/dl)	Basal	0.32(0.14-0.41)	0.78	0.25(0.17-0.36)	0.95	0.25(0.18-0.34)	0.78	0.26(0.16-0.36)	0.81
	6 months	0.26(0.11-0.44)		0.26(0.18-0.37)		0.265(0.18-0.36)		0.25(0.16-0.38)	
	1 year	0.32(0.26-0.48)		0.24(0.20-0.37)		0.26(0.18-0.36)		0.25(0.20-0.37)	
Urea (mg/dl)	Basal	21.35(16.65-26.80)	0.664	19.95(15-26.63)	0.370	20(16-28)	0.07	20.20(15.00-25.70)	0.88
	6 months	19.25(15.00-22.83)		19.00(13.60-23.00)		16.5(12.98-21.55)		19.30(15.20-23.25)	
	1 year	18.45(14.65-26.08)		20.60(15-24.60)		16.60(13.05-23.10)		20.55(15.30-24.80)	
Creatinine (mg/dl)	Basal	0.63(0.45-0.78)	0.26	0.44(0.32-0.58)	0.0006***	0.40(0.30-0.56)	0.173	0.48(0.34-0.63)	0.0011**
	6 months	0.50(0.24-0.61)		0.38(0.33-0.45)		0.37(0.33-0.42)		0.39(0.33-0.49)	
	1 year	0.45(0.30-0.50)		0.37(0.32-0.44)		0.35(0.305-0.405)		0.39(0.34-0.49)	
Amylase (U/L)	Basal	69.5(46.7-115.8)	0.38	78.5(56-102)	0.29	82(53-107)	0.66	77(56-101)	0.57
	6 months	65.50(46.7-115.8)		77(53-101)		70.5(52.5-104)		79(51.50-101)	
	1 year	82.5(63.2-143.8)		74(51-96)		72(58-87.5)		78.50(49-98.75)	

Platelet (Lakhs)	Basal	291000(170750-326250)		288000(221000-320500)		283000(218000-312000)		289000(222000-325000)	
	6 months	299500(235750-310000)	0.650	294000(239000-310000)	0.088	296000(258750-310500)	0.225	294000(233500-310000)	0.153
	1 year	296500(295250-300750)		306000(281000-314000)		304000(283500-312000)		302000(280000-314500)	

ANOVA test, P<0.05 considered significant

Majority of the non-responders were of wild type in patients with A17G and C133T. Whereas for other genotypes (T31C, G81A, T140C, A477G). Majority of the non-responders were of mutant alleles (Table 11)

Table 11: Pattern of UGT1A6 Gene polymorphism in non-responders (N=20)

	Pattern	Frequency	% of patients
A17G	AA	19	95
	AG	1	5
	GG	0	-
T31C	TT	0	-
	TC	11	55
	CC	9	45
G81A	GG	4	20
	GA	15	75
	AA	1	5
C133T	CC	20	100
	CT	-	-
	TT	-	-
T140C	TT	1	5
	TC	18	90
	CC	1	5
A477G	AA	4	20
	AG	16	80
	GG	-	-

Table 12: Comparison of sodium valproate drug concentration with clinical outcome

Clinical outcome	Basal (µg/ml)	6 months (µg/ml)	1 year (µg/ml)	P value

Responders	101.4±34.23	92.82±42.21	78.52±35.00	0.0011**
Non- Responders	111±33.35	87.16±41.97	73.89±39.69	0.0481*

ANOVA test, $p<0.05$ considered significant

Except for one patient who developed acute pancreatitis, none of them had any adverse effects. Three patients had an asymptomatic increase in SGOT, and two patients had asymptomatic increased ALP levels. All children had normal liver function parameters, hematological and renal function parameters during basal and 1-year follow-up. One patient developed acute pancreatitis with elevated serum lipase and featured suggestive of pancreatitis in USG. De-challenge was given to this patient, and the patient recovered completely within a week. The patient was shifted to levetiracetam.

The Naranjo scale contains ten questions which are designed to obtain information on various aspects of adverse drug reaction and the medications that the patient is receiving. These questions will seek responses as 'yes'/'no'/'do not know'. The adverse drug reaction is assigned to a probability category of 'definite ADR' if the total score is ≥9 and 'probable ADR' if the total score is between 5 – 8. 'Possible ADR' is assigned to the score between 1 – 4 and 'doubtful ADR' for the score of 0.

The Naranjo Algorithm is a questionnaire designed by Naranjo et al for determining whether an ADR (adverse drug reaction) is actually due to the drug or due to other factors. Values obtained from the algorithm are sometimes used in peer reviews to verify the validity of author's conclusions regarding adverse drug reactions. Causality assessment was performed using the Naranjo algorithm by Senior Research Fellow under the supervision of treating pediatrician. The Naranjo score of this particular adverse drug reaction (Acute pancreatitis) found to have a score of +6, suggesting the causality of ADR as 'Probable'.(Table 13)

Table 13A: Causality assessment by Naranjo scale

Questions	**Yes**	**No**	**Do not know**	**Assessment Score**
Are there previous conclusive reports on this reaction?	+1	0	0	**0**
Did the adverse event appear after the suspected drug was administered?	+2	-1	0	**+2**
Did the adverse reaction improve when the drug was discontinued, or a specific antagonist was administered?	+1	0	0	**+1**
Did the adverse event reappear when the drug was re-administered?	+2	-1	0	**0**
Are there alternative causes (other than the drug) that could on their own have caused the reaction?	-1	+2	0	**+2**
Did the reaction reappear when a placebo was given?	-1	+1	0	**0**
Was the drug detected in blood (or other fluids) in concentrations known to be toxic?	+1	0	0	**0**
Was the reaction more severe when the dose was increased or less severe when the dose was increased?	+1	0	0	**0**
Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0	**0**
Did any objective evidence confirm the adverse event?	+1	0	0	**+1**
Total Score:				**6**

Table 13B: Reply to the framed questions of Naranjo Scale:

Questions	**Reply**	**Assessment Score**
Are there previous conclusive reports on this reaction?	No	**0**
Did the adverse event appear after the	Adverse event appeared after initiation of	**+2**

suspected drug was administered?	Sodium valproate treatment.	
Did the adverse reaction improve when the drug was discontinued, or a specific antagonist was administered?	Adverse event improved when treatment was discontinued. No specific antagonist was administered. Levetiracetam treatment was started.	**+1**
Did the adverse event reappear when the drug was re-administered?	Drug was not re-administered again.	**0**
Are there alternative causes (other than the drug) that could on their own have caused the reaction?	No	**+2**
Did the reaction reappear when a placebo was given?	Do not know, because placebo was not prescribed.	**0**
Was the drug detected in blood (or other fluids) in concentrations known to be toxic?	Drug was not found to be in toxic range.	**0**
Was the reaction more severe when the dose was increased or less severe when the dose was increased?	Rechallenge was not done.	**0**
Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	No	**0**
Did any objective evidence confirm the adverse event?	Biochemical and ultrasonography finding were suggestive of acute pancreatitis. **Brief report:** Patient was on Sodium Valproate treatment (T. Enchorate Chrono 500mg BD) since Day 1 Developed pain abdomen, more in peri-umbilical region, from Day 5 USG was done on Day 12 Impression: Reactive mesenteric lymphadenopathy, Appendix was normal, Head of pancreas – Bulky. De-challenge was done on Day 13, and the patient recovered completely within a week. The patient was shifted to levetiracetam. On further follow up visit, patient was doing well with levetiracetam.	**+1**

Biochemical parameters	Normal range	Day 1	Day 5	Day 12	Day 13
Lipase	13-60 U/L		152 U/L	54 U/L	67.1 U/L
Amylase	20-160 U/L		122 U/L	84 U/L	84 U/L
SGOT	0-45 U/L	29 U/L	74 U/L	163 U/L	112 U/L
SGPT	0-45 U/L	35 U/L	72 U/L	140 U/L	106 U/L
Sod. Valproate	50-100 **µg/ml**	90 **µg/ml**	55 **µg/ml**		

Total Score:	**6**

Correlation study showed that only creatinine levels showed significant (p=0.011) negative correlation with sodium valproate concentration at the basal level, other parameters like albumin, total protein, bilirubin direct, bilirubin total, alkaline phosphatase, amylase, and platelet also bear an insignificant negative correlation. An

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.inj14
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

insignificant positive correlation was noted between basal sodium valproate concentration and SGPT, SGOT, and urea. (Table 14)

At six months follow-up, a negative correlation was observed between sodium valproate concentration and albumin, total protein, SGPT, bilirubin direct, bilirubin total, creatinine, and amylase; however, the correlation was insignificant. An only significant negative correlation was observed between sodium valproate and platelets at 6-month follow-up (p=0.023). At six months of follow-up, sodium valproate was found to bear an insignificant positive correlation with SGOT, alkaline phosphate, and urea. (Table 14)

A significant correlation was observed at one year of follow-up between sodium valproate and SGPT, urea, and amylase (p=0.005, 0.049, 0.020 respectively); however, other parameters did not have a statistically significant correlation at one year of follow-up. (Table 14)

Table 14: Correlation between Sodium valproate concentration and biochemical and hematological parameters.

	Basal		Six months		One year	
	r-value	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**
Albumin (g/dL)	-0.054	0.592	-0.056	0.633	0.113	0.347
Tot prot (g/dL)	-0.024	0.810	-0.052	0.660	-0.005	0.969
SGOT (IU/L)	0.082	0.416	0.043	0.711	0.158	0.189
SGPT (IU/L)	0.052	0.604	-0.052	0.655	0.327	**0.005***
ALP (IU/L)	-0.112	0.268	0.074	0.530	0.156	0.194
BID (mg/dL)	-0.026	0.795	-0.120	0.304	-0.043	0.724
BIT (mg/dL)	-0.070	0.490	-0.090	0.440	-0.076	0.528
Urea (mg/dL)	0.006	0.951	0.068	0.561	0.235	**0.049***
Creatinine (mg/dL)	-0.254	**0.011***	-0.161	0.168	0.096	0.425
Amylase (IU/L)	-0.124	0.218	-0.044	0.711	0.276	**0.020***
Platelet (lakh/mm^3)	-0.080	0.428	-0.262	**0.023***	-0.131	0.277

Pearson correlation coefficient test used.
*P-value <0.05 considered statistically significant

ROC has been constructed to assess the utility of sodium valproate concentration in assessing the outcome in terms of seizure frequency. The area under the curve (AUC) was 0.587, with a sensitivity of 60 % and specificity of 50 %. The cut-off value of sodium valproate was noted to be 107.5 µg/ml. These findings suggest that sodium valproate concentration may not be a sole marker for predicting clinical outcomes (Fig 2).

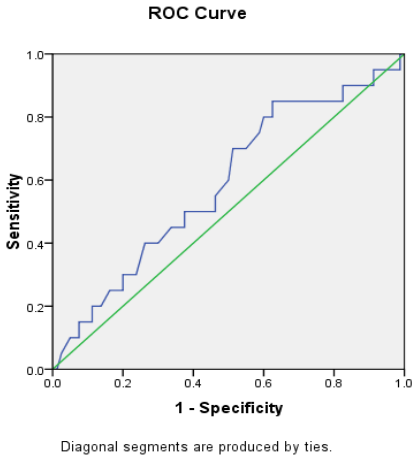

Fig 2: ROC for Utility of VPA to predict the clinical outcome

Discussion

The liver, as well as the biliary and gastrointestinal tracts, are the primary sites of UGT1A3 expression. The

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.inj14
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

UGT1A3 gene has a high rate of mutation, and numerous mutation sites have been found. There are now 31 single nucleotide polymorphisms (SNPs) in the UGT1A3 gene's promoter region and first exon, most of which are brought on by alkaline substitutions [22].

In the present study, almost there was no mutation for A17G and C133 T, whereas majority of the alleles were of mutant type in rest of the four mutations (table 2). Studies have shown that mutations are identified in four (i.e., T31C, G81A, A477G, and A17G) out 7 loci of UGT1A3, while no mutation is revealed in other three loci (i.e., C133T, A808G, and G342A) [22].

There was no significant association between gene polymorphisms and serum VPA concentrations, though VPA levels were significantly different in different intervals (table 6).

When compared to homozygotes with only the T31C mutation in the wild type, the standardised plasma concentration of sodium valproate in children with heterozygous mutations is considerably lower. The conclusion drawn from the high allele frequency of T31C (36.67%) is that this genetic polymorphism has a significant impact on the plasma concentration of sodium valproate in children with epilepsy, and that children who have heterozygous mutations should receive higher doses of sodium valproate to achieve the target plasma concentration range [22]. These results, which demonstrate that the UGT1A3 gene can influence the activity of its own transcription enzymes, are in line with those shown by Cho et al. [23]. The small sample size of the study and the variations in drug metabolism between adults and children may be to blame for the inconsistent nature of these findings. Studies on UGT1A3's effects on AEDs are still scarce, and further research is required to determine how UGT1A3 contributes to changes in medication plasma concentration.

Mutations would have been beneficial for A17G, C133T but dangerous in T31C, G81A, T140C and A477G (table 4). However, the observed alleles were helpful in metabolizing VPA (as represented by the proportion of responders) (tables 4,7,8). There was no significant association between polymorphism and seizure type (table 9).

UGT1A3 is an enzyme that glucuronidates xenobiotic and endobiotic compounds. A Japanese study by Iwai et al. found polymorphisms of this enzyme, and we discovered that exon 1 of UGT1A3 produced from the DNA of 100 healthy Japanese volunteers included six SNPs [24]. Two of them were silent alterations at codons 27 and 159, while four of them (Q6R, W11R, W45R, and V47A) led to amino acid modifications. SNP occurrences reveal the existence of polymorphisms, some of which contain a mixture of SNPs. In the population sample under study, nucleotide sequencing identified five polymorphisms that encoded for five different forms of UGT1A3 proteins. The enzyme with the most common wild type allele for UGT1A3 has the following amino acid sequences: Q6-W11-R45-V47. In the study, 36% of the population was homozygous for the wild form of UGT1A3. They discovered two parallel SNP pairings, Q6A-W11R and W11R-V47A, which result in amino acid alterations on single alleles.

We have not observed patients with gastrointestinal disturbances like nausea, vomiting, abdominal pain, etc. Our study population did not have transient hair loss or change in hair color or texture during therapy. An insignificant weight gain was observed in some subjects, which was not statistically significant. None of the study participants were observed with altered sleep patterns or duration of sleep or child feeling drowsy during the therapy. Skin rashes were also not observed in patients who were under treatment.

Except for two patients, all children had normal liver function, hematological and renal function parameters during basal and 1-year follow-up. One patient developed acute pancreatitis with elevated serum lipase and featured suggestive of pancreatitis in USG. De-challenge was given, and the patient recovered completely. No challenge was given and the patient was shifted to levetiracetam. Causality assessment was done using the Naranjo algorithm, which revealed a score of +6, suggesting the probable nature of ADR due to sodium valproate.

In our study population, sodium valproate efficiently controlled seizures with good tolerability. Treatment adherence may be improved by educating parents and other patient carers about various adverse drug effects, their early detection, management, and preventative measures. The earlier these pharmacological side effects are identified and managed, the easier it will be to negotiate them, get good results, and generally improve the patient's quality of life.

The discrepancy between our findings and those examined in different other groups may be partially explained by differences in methodology, ethnicity, sample collection methods, drug concentration estimate techniques, sample sizes, and phenotypic definitions like drug responsiveness.

Sample size restriction is a significant and frequent problem in candidate gene association research, including the majority of the studies previously cited as well as our own. Since children often have considerably higher sodium valproate metabolism than adults, our study primarily focused on paediatric epileptic patients.

The human liver exhibits the highest activity of the uridine diphosphate glucuronidase (UGT), which is broadly distributed in many human organs and tissues, including the kidney, heart, brain, and skin [25,26]. UGT is primarily found in the liver and has a diverse range of substrates. It is responsible for both catalysing the metabolic reactions of exogenous substances like drugs (such as CBZ, valproate, non-steroidal anti-inflammatory drugs, morphine, zidovudine, chloramphenicol, and farnesol) and endogenous substances like steroid hormones, bile acid, and tretinoin [27].

UGT, a crucial enzyme involved in the phase II metabolic response in the human body, makes its metabolic substrates more water-soluble by glucuronidation, thus aiding in their excretion. The UGT gene is polymorphic, which results in the encoding of enzymes with different rates and levels of substrate metabolism [28]. The UGT genes are mostly found in two families in humans: UGT1 contains the subfamily UGT1A and UGT2 contains the subfamilies UGT2A and UGT2B [29].

No matter whether the epilepsy patients were treated concurrently with VPA and other antiepileptic medicines or VPA monotherapy, the genotype frequencies of UGT1A3 A17G, T31C, G81A, C133T, T140C, and A477G in previous study [30] were similar to our research. Additionally, they demonstrated a substantial impact of the UGT1A3 A17G polymorphism on plasma levels of VPA in Chinese patients with epilepsy, with greater VAP Cs in carriers of the AA genotype than in carriers of the AG genotype.

The UGT 1A3 isoform has received the least research attention. The most extensively researched polymorphs were UGT1A6 and UGT 2B7.
Genetic testing has progressively grown in importance as a medical pillar in the clinical use of AEDs to treat patients with epilepsy, offering therapeutic direction for the selection of clinical AEDs.

The determination of key sites for drug-metabolizing enzymes and transporter genes is crucial and useful to effectively analyze and predict the response of various patients to various types of AEDs, ultimately leading to the establishment of the individualized doses, based on the metabolic characteristics of both traditional and new AEDs. Individualized drug delivery for epilepsy patients would make it easier to quickly and precisely determine the treatment window, prevent drug waste, ease financial strain, choose the right drug type and dosage with the fewest side effects, and select AEDs that are difficult to tolerated to maintain long-term curative effects. Thus, the study and discovery of the genes encoding the drug-metabolizing enzymes is crucial for the clinical management of epilepsy.

To present, many significant hospitals in China have employed the identification of drug-metabolizing genes extensively in the clinical management of epilepsy. Patients' oral mucosal epithelial cells are routinely sampled for DNA to identify target genes, which is then used to select appropriate AEDs for patients with low risk of side effects and high level of bioavailability based on the phenotypes of various target genes. These findings make it much easier to build a distinctive genetic database of the Chinese population and, ultimately, to give clinical advice specific to the Chinese population based on the traits of genetic distribution. Additionally, the identification and collection of a significant amount of data offer solid theoretical and experimental support for the creation and fabrication of novel AEDs. Based on the easy collections of oral mucosal epithelial cells, it is envisaged that in the future it will be extremely likely to provide thorough recommendations of the types, doses, and frequency of administration of the personalized AEDs.

Epilepsy is a serious condition that has a negative influence on human health, and research into effective therapies is ongoing globally. Despite having significant adverse effects, conventional AEDs are nevertheless frequently used in the present treatment of epilepsy.

The clinical understanding of how genetic variation in the genes that code for drug-metabolizing enzymes affects the plasma concentration of AEDs has greatly increased. It is becoming more and more important to condense and conceptualize the clinical importance of current studies on customized treatment plans. Our investigation provides strong experimental support for the development of clinical recommendations for the use of AEDs.

Conclusion

- There was a higher pattern of mutant carrier alleles for the gene UGT T31C, G81A, T140C and A477G.
- There was no association between sodium valproate concentration with gene UGT1A3 genotypes studied, although the mean concentration of sodium valproate was high in wild types compared to mutant types.
- Different patterns of UGT1A3 gene polymorphism did not show any significant association with the clinical outcome of Sodium valproate in terms of efficacy and tolerability of epilepsy.
- UGT1A3 gene polymorphism studies may be used before initiating sodium valproate treatment to predict the treatment response.
- The drug was well tolerated in our study population without much change in hepatic, renal, and pancreatic parameters. Sodium valproate was well-tolerated among pediatric patients with epilepsy and can be used as an effective anti-epileptic drug.

Funding Agency: Indian council of medical research

Acknowledgements: Central research laboratory, KSHEMA

Conflicts of interest: None

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