

Evaluation of sodium valproate levels in malnourished childhood epilepsy and its consequences

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ABSTRACT

Objectives. To measure and compare free fraction of serum levels of VPA (valproic acid) between non-malnourished and malnourished epileptic children and to evaluate the adverse effects (myopathy, hepatotoxicity).

Material and methods. It is a prospective comparative observational case control study in which forty epileptic children (malnourished: 18 male/8 female, age 8.3±2.5, non-malnourished: 8 male/6 female, age 8.1±2.1) who fulfilled the inclusion criteria were recruited as study group and twenty children as control group (12male/8 female, age 6.0±2.8). Outcome measures monitored are serum VPA levels (total and free fraction of serum VPA), serum CK (creatinine kinase), SGOT (serum glutamic oxaloacetic transaminase) and SGPT (serum glutamic pyruvic-transaminase). Using screening tool for the assessment of malnutrition in pediatrics (STAMP©), subjects are categorized into mild, moderate, severe malnourished as -1SD, -2SD, -3SD respectively

Outcomes. There is an elevation in mean free fraction of VPA is 17.3±6.4 µg/ml whereas in non-malnourished group it was found to be 8.7±4.2 µg/ml with P= <0.001. While mean total drug concentration in malnourished and non-malnourished was found to be 88.6±49.9 µg/ml, 70.8±33.9 µg/ml respectively. Mean CK, SGOT, SGPT in control is 29.3±9.9 IU/L, 28.5±5.4 IU/L, 24.5±4.2 IU/L respectively. whereas mean CK, SGOT, SGPT in malnourished subjects is 16.9±9.1 IU/L, 28.8±8.5 IU/L, 25.9±9.1 IU/L Correspondingly .while Mean CK, SGOT, SGPT in non-malnourished individuals is 15.7±11.3 IU/L, 32.4±8.8 IU/L, 28.7±7.8 IU/L respectively. No correlation was observed between elevated serum drug concentrations, clinical response and side effects.

Conclusion. We observed that clinical response and side effects are serum concentration independent hence our research make unnecessary to monitor serum drug concentration for every individual and drug monitoring should be restricted to subjects with severe ADR's. Our data also suggest Valproate unveiled mitochondrial myopathy is limited to subgroup of population.

Keywords: sodium valproate, malnutrition, adverse effects, free fraction of drug

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INTRODUCTION

Sodium valproate is the most commonly used drug in pediatrics with epilepsy because of its low toxic profile and broad spectrum of activity against different kind of seizures (1). It is a highly protein bound (78-94%) with time to peak level of 3-6

hours, half-life of about 11-20 hours and time to attain steady state concentration of 2-4 days (2). There is a decrease in plasma proteins which is a common feature of hypoproteinemia in malnourished children. (3) which in turn increases volume of distribution (Vd) of sodium valproate.

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Therapeutic drug monitoring (TDM) studies have demonstrated no correlation between dose administered with serum levels of VPA and also with clinical response (4,5). Toxic effects like sedation, depigmentation of hair and hair loss were seen when the serum VPA levels exceed 600 $\mu\text{mol/l}$ (5). In a VPA intoxication case report there is an escalation of SGOT, SGPT and CK in whom plasma levels of VPA was found to be very high (9064.4 $\mu\text{mol/L}$) (6). There is a deficiency of data correlation between serum VPA levels SGOT and SGPT levels, our work is aimed to fulfill this deficiency.

It is well known that sodium valproate is an enzyme inducer and it cause escalation of SGOT, SGPT levels. Zimmerman et al. has reported valproate induced hepatotoxicity in twenty three patients which shows an elevation in SGOT and SGPT levels which is due to metabolites (7). In a review by Jeavons et al. have reported hepatotoxicity in eighty eight patients who are on sodium valproate which is most likely to appear within six months on start of therapy (8).

A review by Pasnoor et al. have shown the possible drugs and their pathologic mechanism's effecting myopathy (9). Various review articles also delineated about drug induced myopathies which have stated that there is a marked elevation of serum CK in myopathy (10-12). Some case reports show elevation of serum CK on treatment with sodium valproate. Some of them suggest it is because sodium valproate triggers the carnitine type-2 deficiency which is the transporter of long chain fatty acids in to the inner membrane of mitochondria which leads to energy depletion in mitochondria of muscle cells by which muscle cells are subjected to rhabdomyolysis in which elevation of CK is seen (13,14). Since there is an increase of free fraction of VPA in malnourished condition free VPA levels may show severe impact on carnitine levels that may increase the risk of myopathy. A case report by Chaudhry, et al. has shown elevation of CK in a 12 year old boy with MELAS (Mitochondrial Myopathy, Encephalopathy, Lactic acidosis and Stroke like episodes) (15). A study in forty one epileptic children shown a significant decrease in serum carnitine levels which does not correlate with SGOT and SGPT levels (16). Similar study in 50 children have shown no correlation between serum VPA levels and serum carnitine levels but have shown

significant correlation between VPA levels and muscle carnitine levels (17). Three case reports were also discussed by Uppin, Megha S. et al. which states that among two patients there is an improvement in serum CK levels on treatment with levocarnitine (18). A case report has shown elevation of CK due to sodium valproate overdose, (6) which may happen in malnourished children due to increase in fraction of free drug concentration in plasma.

We believe in the hypothesis that free drug concentration of valproate is going to increase in malnourished patients which in turn effects the enzymes like CK, SGOT and SGPT. Because of the scarcity of data about VPA concentration in malnutrition population whose prevalence is more in countries like India our work is focused to fulfill the scarcity and safety monitoring in this subset population. Hence the present work is aimed to study the effect of malnutrition on drug concentration and to study how the elevated drug concentration will affect the CK, SGOT and SGPT and finally to compare the results obtained between healthy and malnutrition patient.

MATERIAL AND METHODS

Subjects

This is a prospective comparative case control study. Control group was included because deficiency of sufficient data on normal range of CK in Indian pediatric population. Study was conducted at Mahatma Gandhi Memorial Hospital which is a 1,000 bedded teaching government hospital and is one of the biggest hospital in Telangana state. Patients who meet the inclusion criteria were recruited for a period of two months that is in the month of August and September of 2016 from pediatric inpatient department.

Subjects who are diagnosed with epilepsy by the physician and who are on sodium valproate 400 mg/day for a minimum of one month were recruited for the study as per the guidelines of declaration of Helsinki. Using screening tool for the assessment of malnutrition in pediatrics (STAMP[®]), subjects are categorized into mild, moderate, severe malnourished as -1SD, -2SD, -3SD respectively. Subject details were collected in data collection form which includes age, gender, anthropometric

parameters, duration of treatment, number of seizure episodes, side effects experienced if any and list of other medications.

Inform consent form is signed from the guardians of the subjects. Samples were collected from the venous blood (2 ml from each participant) after 3 hours of oral administration, however subjects with recent seizure episodes were excluded from the study because of elevated CK which may last 3 to 8 days (19) number of seizure episodes were tracked by interviewing the guardians of children and adverse effects are monitored by examining present and past medication charts of the patients. Subjects with conditions like hypothyroidism, hyperthyroidism, who underwent recent surgery or trauma, patients on other myopathy inducing drug (except study drugs), hepatic or renal disease, (20) patients who are newly prescribed on sodium valproate were also excluded. Samples were collected in to Eppendorf tubes which are centrifuged at 4,000 rpm and stored at -20°C to measure free fraction of VPA. 1 ml of the blood sample was collected in to amicon tubes and centrifuged at 4,000 rpm for protein separation and obtained clear liquid is stored at -20°C. No deterioration of the samples were seen during the time of analysis. CK, SGOT, SGPT levels were analyzed on the same day of the sample collection whereas serum free fraction VPA levels were measured within one month of sample collection.

Data assessment

Serum levels of free fraction sodium valproate was determined by HPLC-UV method which was developed by kishor et al. (21) equivalent amount of acetonitrile with 2.5 µg diazepam as an internal standard is added to the serum for protein precipitation and mixed on the Cyclomixer which is then centrifuged at 4000 rpm for 10 minutes 20µl of the supernatant is injected using Hamilton injection in to HPLC with running time of 10 minutes. Chromatograms of the individual subjects were recorded and saved. This method was carried out on Shimadzu HPLC using C-18 column at 50° C using acetonitrile and 0.05M phosphate buffer (pH 3.0) 45:55 as a mobile phase at a flow rate of 1.2 ml/min.

Creatinine kinase, SGOT and SGPT levels were determined calorimetrically using Turbochem-100 auto analyzer.

Statistical analysis

Statistical analysis were carried out by using SPSS 20.1 for windows, our data is within the skewness hence analysis were performed on parametric data. Descriptive statistics like mean, standard deviation were calculated for each outcome measurement. Outcome of each measurement was correlated by using Pearson's correlation. Serum VPA levels were compared by two independent sample *t* test by setting level of significance at 0.05.

TABLE 1. Distribution of all variables in control and epilepsy groups

Characteristics	Control (n=20)	Epilepsy patients		P value
		Without malnutrition (n=14)	With malnutrition (n=26)	
Age, mean(SD)	6.0 (2.8)	8.1 (2.1)	8.3 (2.5)	-
Height, mean (SD)	114.8 (15.9)	128.2 (22.0)	119.5 (15.0)	-
Weight, mean (SD)	21.4 (6.8)	28.1 (10.7)	19.8 (5.2)	-
Gender, Male (%)	12 (60.0)	8 (57.1)	18 (69.2)	-
Female (%)	8 (40.0)	6 (42.8)	8 (30.7)	-
Head Circumference, mean (SD)	49.4 (4.6)	50.5 (2.6)	47.5 (8.8)	-
Mid upper arm circumference, mean (SD)	20.7 (4.5)	20.2 (3.2)	16.5 (3.6)	-
Consanguinity, Yes (%)	0 (0)	6 (42.9)	6 (23.1)	-
No (%)	20 (100.0)	8 (57.1)	20 (76.9)	-
Developmental delay, Yes (%)	0 (0)	0 (0.0)	6 (23.1)	-
No (%)	20 (100.0)	14 (100.0)	20 (76.9)	-
Free VPA, mean (SD)	-	8.7 (4.2)	17.3 (6.4)	<0.001**
Total VPA, mean (SD)	-	70.8(33.9)	88.6 (49.9)	0.003
Serum CK. Mean (SD)	29.3 (9.9)	15.7 (11.3)	16.9 (9.1)	< 0.001*
SGOT, mean (SD)	28.5 (5.4)	32.4 (8.8)	28.8 (8.5)	0.187*
SGPT, mean (SD)	24.5 (4.2)	28.7 (7.8)	25.9 (9.1)	0.176*

** Two independent sample *t* test

* One way ANOVA

SGOT, SGPT and CK were compared by one way ANNOVA. Histograms were visually analyzed to conform the distribution of the variables (Table 1).

RESULTS

A total of 76 subjects were screened in the study who are between 2 to 12 years of age, out of whom 20 are control and remaining forty who meet the inclusion criteria were included, among them 26 are with malnutrition and remaining 14 are non-malnutrition. 16 patients who do not meet the requirements of inclusion criteria were excluded. This is illustrated in flowchart 1. Mean age of control group is 6.0 ± 2.8 years whereas mean age in group without malnutrition is 8.1 ± 2.1 years, 8.3 ± 2.5 years in malnourished children.

When compared with non-malnourished subjects peak heights and peak areas are significantly elevated in malnourished individuals which are illustrated in Fig. 1, 2, 3.

No correlation was seen between serum VPA levels and SGOT, SGPT, levels but there is a moderate negative correlation between serum VPA levels and CK ($p < 0.001$).

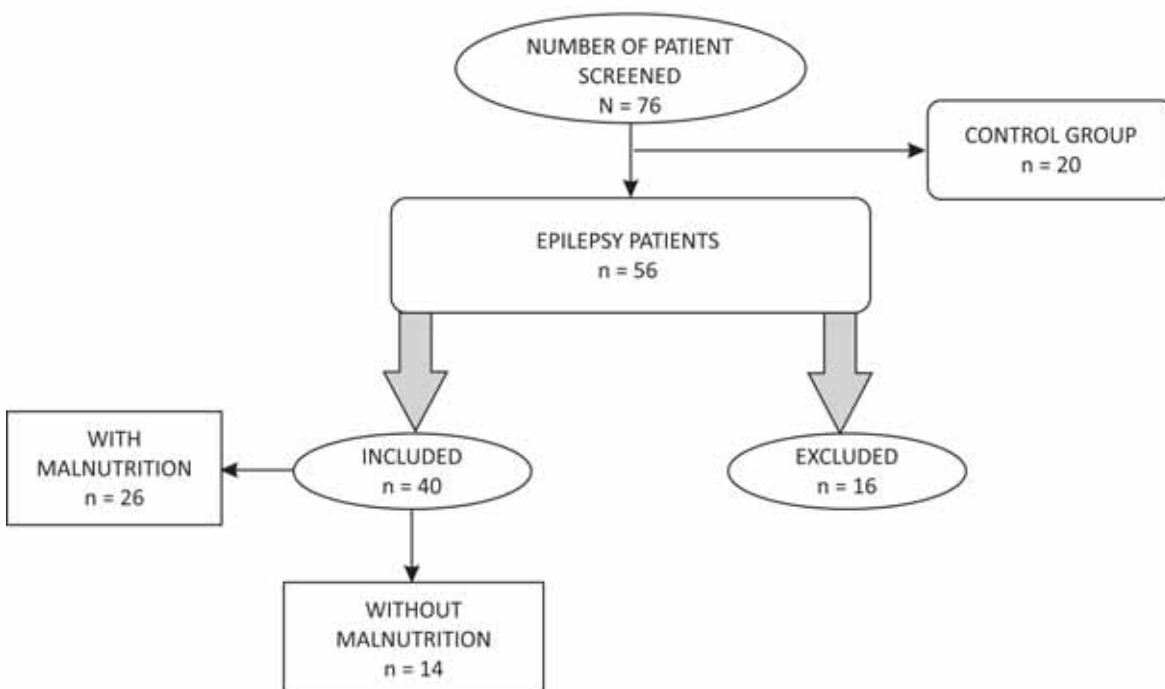
10 non-malnourished and 16 malnourished children have shown an improvement in clinical outcome (decreased number of seizures from the be-

ginning of treatment), while 4 subjects in non-malnourished and 10 in malnourished have experienced recurrence of seizures and experienced poor clinical outcome, which is no decrease in number of seizure frequencies. Among the improved group 6 subjects have their free serum VPA levels in therapeutic range, 12 subjects have sub-therapeutic levels and 8 subjects have above the therapeutic levels of VPA. Among the group with poor clinical outcome 2 subjects have free serum VPA levels within the therapeutic range, 6 are with sub-therapeutic levels and 6 are with above the therapeutic levels.

30 percent of the treatment group experience muscle pains, 10 percent experience headache, 5 percent have complained about weight gain, another 5 percent complained about anorexia while 50 percent of the treatment group did not complained about any symptoms. When duration is considered muscle pain is the prominent complain among the children who are on valproate for longer duration (Fig. 4).

DISCUSSION

Our work determine that clinical outcome is independent to that of serum drug concentration which have been demonstrated in previous studies



Flowchart 1. Recruitment procedure and number of patients in each groups

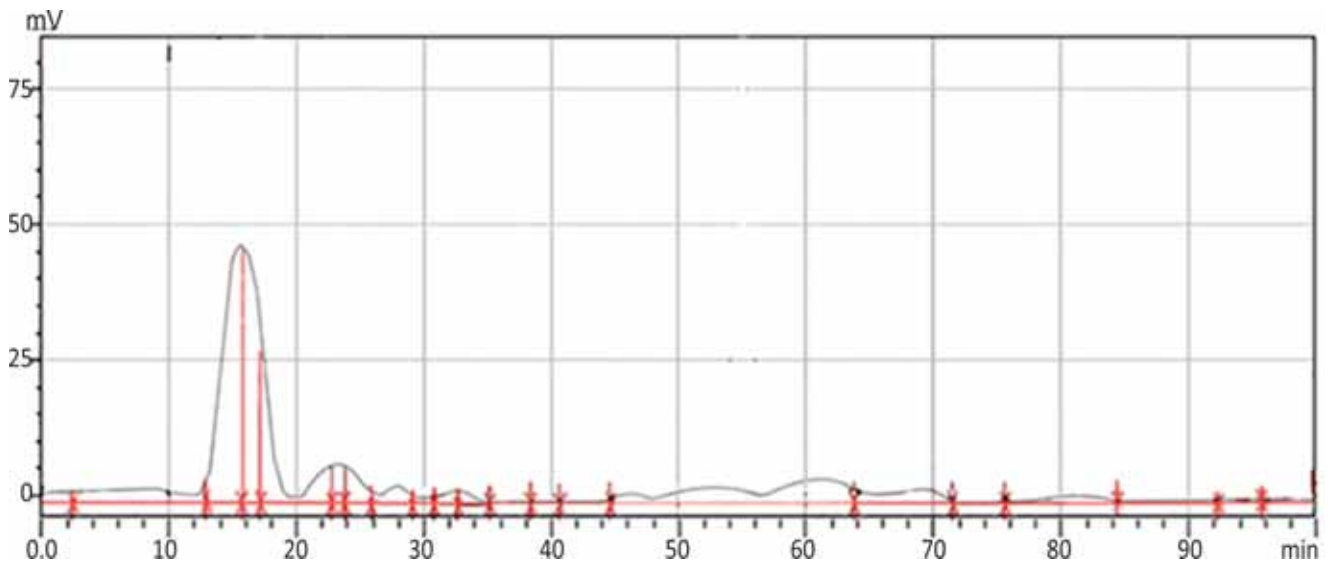


FIGURE 1. Chromatogram of blank serum

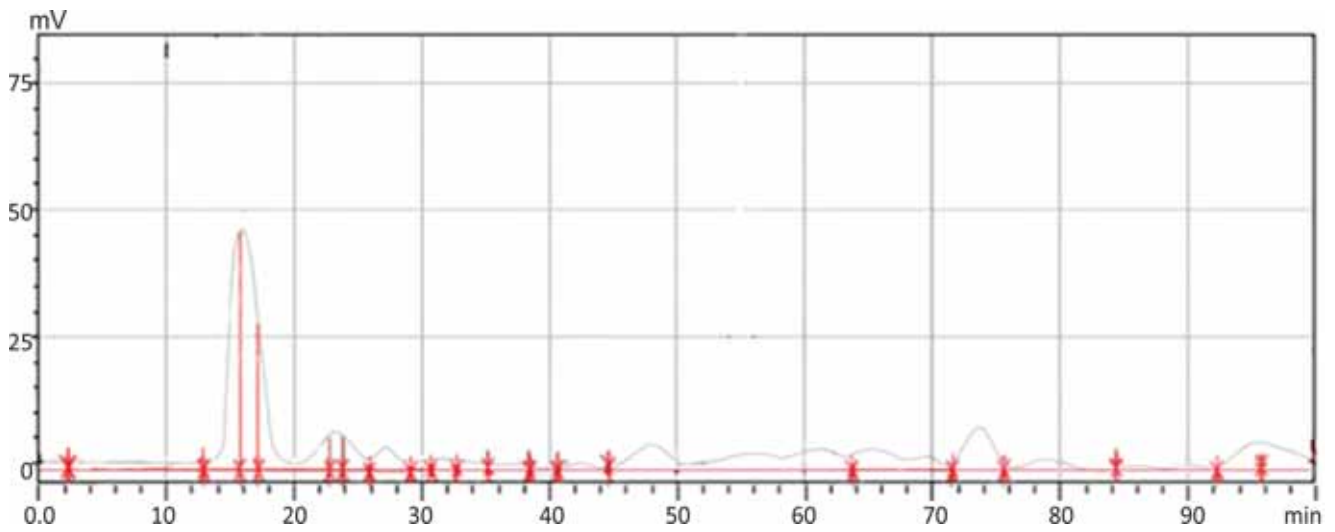


FIGURE 2. Chromatogram of without malnutrition patient

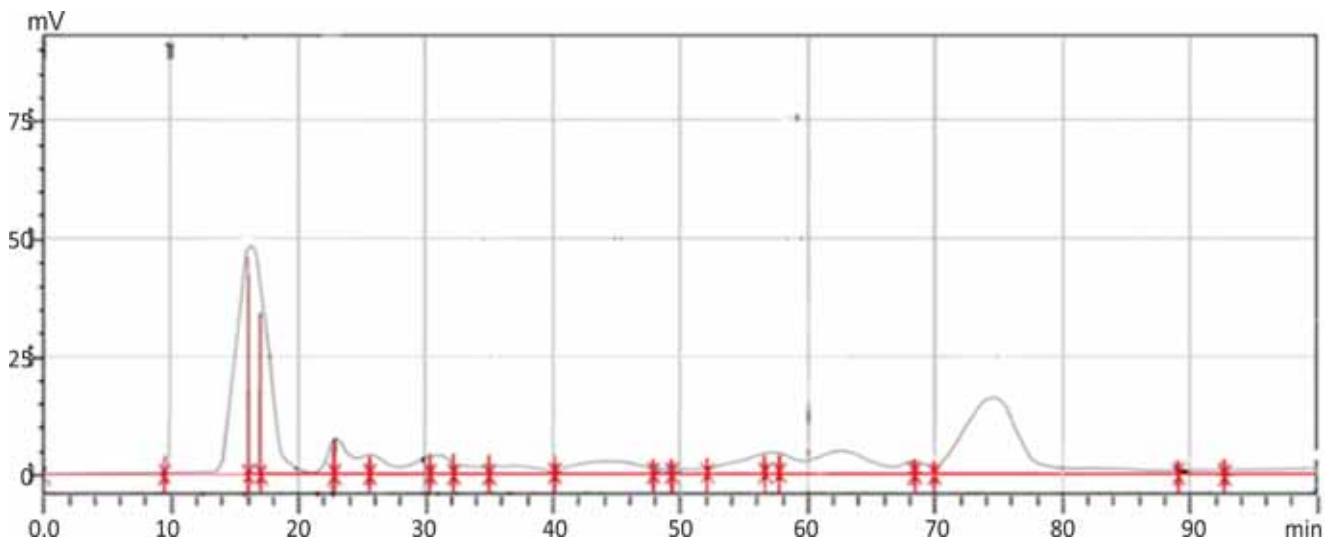


FIGURE 3. Chromatogram of malnourished patient

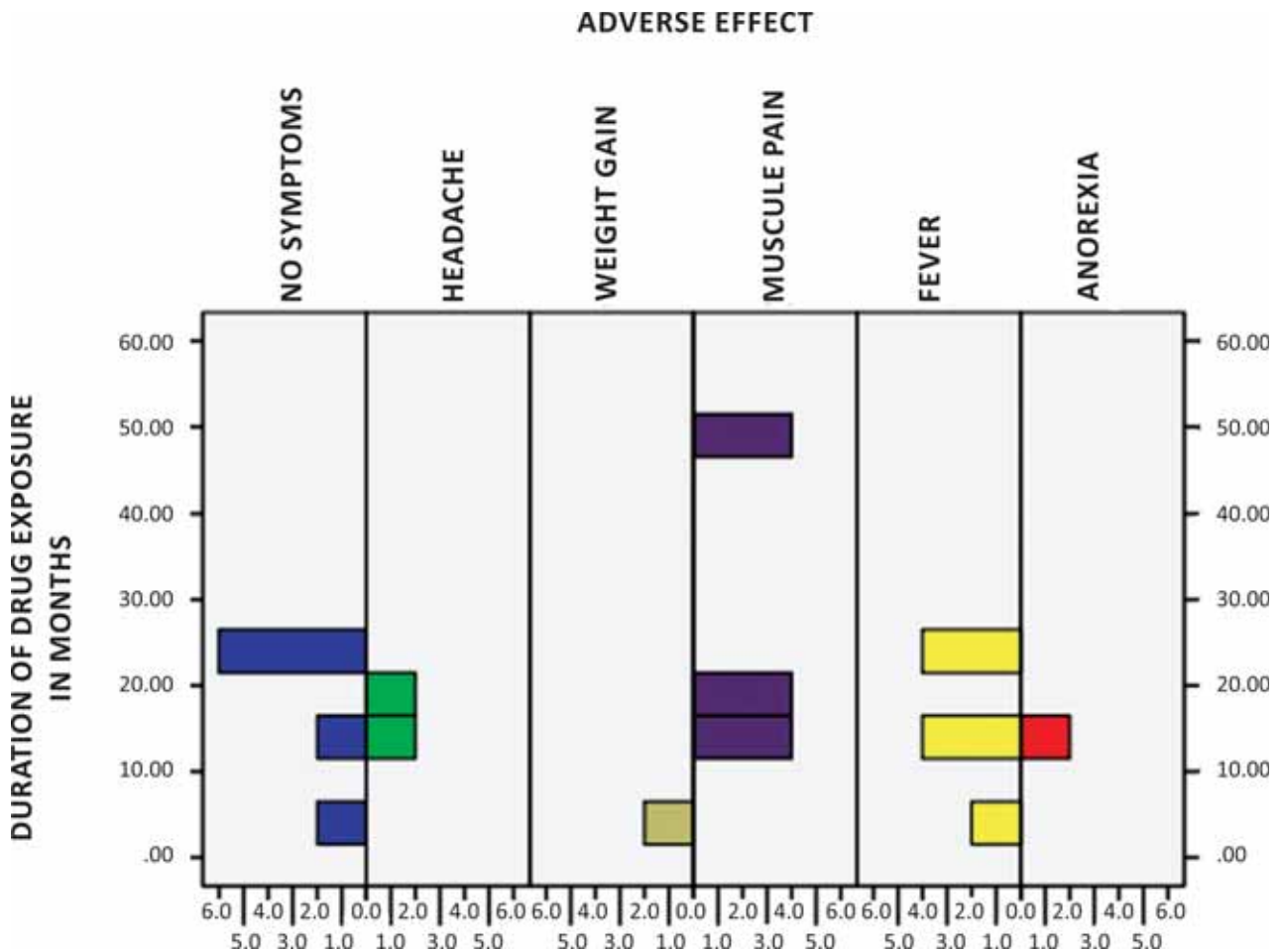


FIGURE 4. Duration with ADRs

(4). Many TDM studies were conducted before but none of them had monitored malnourished pediatric population which is novel in our work. TDM in malnourished children is essential in countries like India where prevalence is more (22). Through which individualization of dose is achieved and side effects are minimized. Although our study spectacle that there is an improvement in seizures we did not find any relation between serum drug levels and clinical control. From HPLC chromatograms we observed escalation of mean free serum VPA in malnourished subjects as predicted. We suspected that side effects are dependent on free serum VPA for which we monitored the SGOT, SGPT and CK levels and correlated with free serum VPA concentration. But our results are contrary to what we expected, no significant correlation was seen between serum VPA concentration with CK, SGOT, SGPT and also side effects.

Our findings espouses that VPA induced myopathy is limited to a subgroup of population (15,23,24). The outcome of our study is contradictory to previ-

ously reported case studies in which there is a rise in serum CK levels in study subjects who were on VPA. But our results show that there is a decrease in mean serum CK levels malnourished group and compared with controlled group, which might be because of pathological changes in malnutrition (25). Nevertheless our results suggest there is a reduction of CK in without malnutrition group which may be due to effect of valproate on muscles.

The possible bias in our study include postictal elevations of serum CK and exercise induced CK elevations which are precluded by excluding such kind of patients (19).

Chaudhary et al. in her case report discussed about mitochondrial myopathy which is unveiled by VPA therapy which had shown that there is an elevation of CK and precipitation of seizures on starting valproate therapy (15). In this report the elevation of CK might not be because of Valproate it might be due to postictal episode. Another case reported by Alexandros et al. had shown CK elevation without seizure episode (26). These cases are

the source of bias that the Valproate therapy is the sole cause for CK elevations.

Limitations of our study include short duration of study, single sample collected and evaluated from the individual participant, lack serum carnitine levels, lack of EMG (Electromyography) and muscle biopsy examinations, which might be needed in further studies to confirm the hypothesis.

Studies in malnutrition children is extensively recommended because of its prevalence in countries like India (22), among whom the possibility of epilepsy is much higher. Kinetic parameters among this population increases the risk of developing drug toxicity by increasing free plasma drug concentration.

In this work we observed an increase in mean free drug concentration in malnourished subjects when compared with healthy individuals. No correlation was seen between elevated free serum drug concentrations, clinical response and side effects hence we conclude that side effects and clinical response is dose independent which was been reported in previous investigations.

CONCLUSION

Our research makes unnecessary to monitor serum drug concentration for every individual and

drug monitoring should be restricted to subjects with severe ADR's or with mitochondrial disorders.

Our data suggest Valproate unveiled mitochondrial myopathy is limited to subgroup of population. It also suggest further studies which include serum drug concentration, biopsy studies, plasma carnitine levels along with CK at multiple intervals in inpatient setup is needed to conform myopathy associated with sodium valproate.

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