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# Therapeutic Drug Monitoring of Levetiracetam by High - Performance Liquid Chromatography in Paediatric Epileptic Patients

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**Abstract:** ***Levetiracetam is a second generation anticonvulsant drug used as adjunctive therapy or monotherapy with high efficacy and tolerability in the treatment of partial seizures, myoclonic seizures and generalized tonic-clonic seizures in children. We aimed to correlate the serum drug concentration with seizure control status, complaints and liver enzymes (Alanine aminotransferase, Aspartate aminotransferase) in pediatric epileptic population.We prospectively evaluated 36 levetiracetam monotherapy patients, the dose was administrated based on their body mass index. A rapid and specific method by high-performance liquid chromatography(HPLC) UV detection was developed to determine serum drug concentrations, observations made and analyzed. Out of 36 patients, 24 patients drug concentration was within therapeutic range (12-46µg/ml) have shown good seizure control,8 patients were in the sub-therapeutic range, of these subjects 4 had good seizure control and another 4 poor seizure control. Remaining 4 patients were in the supra-therapeutic range. This drug has no effect on liver enzymes. There is no significant correlation between serum drug concentration levels and subjective complaints.Levetiracetam can be used as a first-line broad-spectrum antiepileptic drug which is well tolerated and achieves good seizure control.***

**Keywords:** Levetiracetam, seizure control, therapeutic range

## 1. Introduction

Levetiracetam is a second generation anticonvulsant drug used as adjunctive therapy or monotherapy with high efficacy and tolerability in the treatment of partial seizures, myoclonic seizures, and generalized tonic-clonic seizures. Levetiracetam has come to clinical use since 1999 in adults and 2006 in children respectively [1-5].

Pharmacokinetic profile of Levetiracetam is quickly absorbed when taken orally (T max-< 1hour), bioavailability (>95%), protein binding (<10%) and metabolism is usually low and the volume of distribution is 0.5-0.7L/kg. Half life ranges from 6 to 8 hours as it is excreted largely unchanged by kidneys [1,3]. Therapeutic range: 12-46µg/ml [1].

Mechanism of action of Levetiracetam is unique where it binds to synaptic vesicle protein (SV2A), a transmembrane protein which involves calcium-dependent exocytosis of synaptic vesicles in the brain which delays nerve conduction and reduces the release of calcium from intraneuronal stores [1].The most common side effects of Levetiracetam include asthenia, headache, somnolence, dizziness, infection [2,3]. Behavioral symptoms like anxiety, irritability, aggression, apathy, and depression [1].

Therapeutic drug monitoring refers to a practice of measuring drug concentration in biological fluids at particular time intervals to maintain the desired concentration and optimize drug therapy. Therapeutic drug monitoring is performed for drugs with a narrow therapeutic range in clinically challenging situations, co morbidities, poor seizure control, marked inter-individual variability, failure of therapeutic drug response [1, 2].

We aimed to correlate the serum drug concentration with seizure control status, complaints and liver enzymes (ALT, AST) in pediatric epileptic population.

## 2. Patients and Methods

This prospective clinico-pharmacological study was designed and conducted in the Department of Pediatrics, Mahatma Gandhi Memorial Hospital /Kakatiya Medical College, Warangal. We have conducted our study for a period of one year (February to December). The study included 36 patients on Levetiracetam monotherapy for at least one month (whose parents give consent to participate in the study) considered as study subjects. 16 were male and 20 female. Age of study subjects ranged from 3 yrs -13 yrs, the youngest child was 3 years and oldest child was13 years were treated with two different dosage forms of Levetiracetam. The dose was given accordingly with their

body mass index (BMI). Another 14 patients who are treated with other antiepileptic drugs along with Levetiracetam, critically ill patients were excluded. Parent's Informed consent form taken in accordance with the declaration of Helsinki for IHEC approval was obtained after submission of protocol IHEC MGM/VCOP/PHARM D/V/003/2017, KIEC/KMC/NCT/NIS/2018/PO6.

**Blood sampling**

Frozen, drug-free plasma (blank plasma) for calibration curves was obtained from healthy subjects stored at -20 ºC and thawed at room temperature before use. Blood samples were drawn from each individual to measure the drug concentration after 1 hr and 30 min for oral and intravenous Levetiracetam respectively, transferred into serum separator tubes and centrifuged at 4 ºC at 3000rpm using REMI centrifuge for 10 min. Serum was separated and stored at -20 ºC.

## 3. Analytical Procedure

**Chemicals and apparatus:**

Levetiracetam was procured from Hetero Drugs Limited. Fluconazole (used as internal standard), ammonium acetate, HPLC grade water, and acetonitrile were procured from Sigma-Aldrich, Mumbai.

High-Performance Liquid Chromatographic system (Shimadzu's LC 20AD) typically consists of a 25µl fixed volume injector (Rheodyne). The chromatographic separation of Levetiracetam & Fluconazole (IS) was performed on C18 (4.6mm*250mm, 5µm), column using UV-visible detector SPD-20A.

**Calibration samples:**

Stock solutions of Levetiracetam and Fluconazole were set up by dissolving the appropriate amount in Acetonitrile to yield 1mg/ml drug concentration. The dissolution was accelerated by sonication of the mixture for 10 min. These solutions were stored at -20ºC. By spiking serum samples which are drug-free along with the working solution, calibration samples in the concentration range from 1-75µg/ml were obtained. This calibration range covers the therapeutic concentrations of Levetiracetam in patient samples.

**Sample Extraction:**

To 250µL serum sample previously spiked with an analyte (of varying concentrations 1-75µg/ml) and Fluconazole (internal standard), an equivalent amount of Acetonitrile was added. The mixture was vortexed for 1min on a cyclomixer, which was further centrifuged at 4 ºC at 3000rpm for 10min. Then the supernatant was allowed for filtration through a syringe filter of 0.2µm pore size and injected into the HPLC instrument.

Chromatographic conditions after injection of 20µL of the prepared sample, the separation of Levetiracetam were accomplished by isocratic elution. The mobile phase is a composition of 10 mm ammonium acetate buffer, and the pH of this solution maintained as 5 ± 0.2 by acetic acid and acetonitrile added 50:50v/v respectively, filtered through a 0.45µm filter. The flow rate of the mobile phase was 0.3ml/min with a run time of 15 min. Chromatograph was read at 205 nm wavelength using detector UV-visible SPD-20A. The retention time of Levetiracetam and Fluconazole (internal standard) was 7.8min and 9.2 min respectively.

**Statistical Analysis**

Statistical analysis was carried out by using the software package SPSS 20.0 (SPSS, Inc., Chicago, USA) using Pearson Correlation test. Results are expressed as mean ± standard deviation (SD).

## 4. Results

36 children on Levetiracetam monotherapy were recruited in to the present clinicopharmacological study. Patient details are as follows. Mean age: 7.28 ± 2.98 years, mean weight: 7.28 ± 2.98 Kgs, mean –BMI found to be 19.21±0.99 in normal weight subjects, 17.21±0.79 in underweight subjects and 15.2±0.81 in severe underweight subjects. 28(77.7%) were from rural areas and 08(22.2%) from urban areas.

Overall seizures are differentiated as complex partial seizures 16 (44%), simple partial seizures 12 (33%), generalized tonic-clonic seizures 8(22%). 8 (22.2%) had mental retardation and in 28 (77.7 %)patients developmental milestones were normally achieved.Parents described various complaints in their children as asthenia, headache, somnolence, dizziness, aggressiveness in 22(61.1%), 14(38.8%) patients have nil to minimal complaints. Various EEG abnormalities noted in 20(55.5% ) were generalized spike and wave activity, periodic lateralized epileptic form discharge, abnormal sleep record shows focal epilepsy arising from left hemisphere and becoming generalized, abnormal background activity with generalized epileptic activity.

**Table 1**

Patient demographics and baseline variables

| | |
|---|---|
| Total number of patients | 36 |
| Sex (F/M) | 20/16 |
| Age (years) (mean ± SD) range | 7.28 ± 2.98 |
| Weight (kg) (mean ± SD) range | 19.75 ± 7.20 |
| Height (cm) (mean ± SD) range | 113 ± 19.02 |
| **Body mass index** | |
| Normal weight | 19.21±0.99 |
| Under weight | 17.21±0.79 |
| Severe underweight | 15.2±0.81 |
| **Area of distribution** | |
| Rural | 28 (77.7%) |
| Urban | 08 (22.2%) |
| **Type of seizure** | |
| Complex partial seizures | 16(44%) |
| Simple partial seizures | 12 (33%) |
| Generalized tonic-clonic seizures | 8 (22%) |
| Number of mentally retarded patients | 8 (22%) |
| **Complaints of patients** | |
| With complaints | 22 (61.1%) |
| Without complaints | 14 (38.8%) |
| **EEG recordings** | |
| Normal | 16 (44.4%) |
| Abnormal | 20 (55.5%) |
| **Types of dosage forms** | |
| **Oral** | |
| Tablet | 16 (44.4%) |

| | |
|---|---|
| Syrup | 10 (27.7%) |
| **INTRAVENOUS** | 10 (27.7%) |
| Injection | |

Liver enzymes levels were normal in all patients, mean ALT was 19.17±5.04 U/L, mean AST was 15.00±5.37 U/L and mean Levetiracetam drug concentration was found to be 20.28±16.22 µg/ml. There is no significant correlation between serum drug concentration levels and subjective complaints noted by parents.

The seizure control and drug concentration levels are …….table.2

| Serum Drug Concentration Range | Seizure Status | | Total |
|---|---|---|---|
| | Improved | Not Improved | |
| Sub Therapeutic Range | 4 | 4 | 8 |
| Therapeutic Range | 24 | 0 | 24 |
| Above Therapeutic Range | 2 | 2 | 4 |
| Total | 30 | 6 | 36 |

## 5. Discussion

36 pediatric epilepsy patients on Levetiracetam were taken up for study and Levetiracetam drug concentrations correlated with pediatric seizure control, tolerability and enzyme induction.

All children with good seizure control were found have Levetiracetam levels in mean therapeutic range. In half of the children with poor seizure control, Levetiracetam levels were in subtherapeutic range. Another half children with sub therapeutic levels of drug shown reasonable control.Two children who had supra therapeutic range Levetiracetam levels well tolerated the drug. There were no increased adverse effects. In the previous found similar tolerance to Levetiracetam in large doses and difficult refractory epilepsy [3].

Various EEG abnormalities like generalized spike and wave activity, periodic lateralized epileptic form discharge, abnormal sleep record showing focal epilepsy arising from left hemisphere and becoming generalized, abnormal background activity with generalized epileptic activity were noted in half of the study subjects.It to note that clinical seizure control was observed in more than 24(66.6%) subjects. Abnormal electrical activity still persistent in 8(22.2%) of these 24 subjects with apparent clinical control and required dose modification.

In the previous study have demonstrated normalization of various EEG abnormalities. A similar finding is noted in the present study[1].

In the previous study seizure freedom and more than 50% reduction in seizure frequency[5].41% decrease in seizure frequency in pediatric epileptic patients was observed[4]. In another study 13 became seizure free even though they had been treated with several anti epileptic drugs prior to Levetiracetam [3].In the present study the seizure reduction in 66.6% subjects.

In a previous study by the seizure control appears to be not influenced in mentally retarded children, in our study also the serum concentrations of Levetiracetam were similar in children with normal and delayed developmental milestones [2].

The mean drug concentration was [20.28±16.22µg/ml], which has no straight forward correlation with therapeutic efficacy and side effects, Frederique Lancelin *et.al*., (6-±18µg/ml), state that for a clinician this is useful in two way to identify sever intoxification (over dosage). He estimated that in 1/3/ to 1/2 poor compliance can be determined, in cases of poor seizure control and refractory epilepsy[1].

Typically, a daily dose of 10mg/kg in two divided doses is given, which is increased up to 60 mg/kgevery 1 to 2 weeks[5]. The dose of 20-25mg/kg/day was used in the present study.

European union with a dose of 20mg/kg minimum and up to more than 60mg/kg high dose intravenous Levetiracetam have shown good results. A biphasic response with low dose and high doses respectively is a known phenomenon with intravenous Levetiracetam [3].

There was no liver enzyme induction in the study subjects at different serum levels. Similar observations were made in the animal studies they further observed normal histopathology of internal organs [12].

In current study 61% patients have complained various side effects headache, somnolence, feeling of weakness and behavioral changes like excessive sleepiness and aggressiveness. In previous study behavioral side effects including violent behavior, restlessness, impulsive behavior (12%), (30%) showed other side effects [5].

Levetiracetam is generally used in refractory epilepsy children with poor seizure control on different AED'S. Addition of levetiracetam has improved seizure control in study[3]. In the present study Levitiracetam is given has initial drug in emergency room for SE patients and orally for long term control as initial and single drug.

In the previous study found lack of efficacy in 15.9% and adverse events in 6% subjects which led to discontinuation of Levetiracetam in their study [2]. In the present study serious ADR to discontinue Levitiracetam were not found. Thus side effects like headache, somnolence were observed in our study these side effects were transient and settled in few weeks. This didn't require discontinuation of Levetiracetam.

There were no significant drug interactions of Levetiracetam with other AED'S in previous study [1]. In the current study there were no additional drug interactions with change of antiepileptic drugs.

**Limitations**

- When multiple drugs were given, drug levels were not performed for other drugs.
- It is an observational study comparison with similar anti-epileptic drugs (or) randomized control trials are preferable.

## 6. Conclusion

Levetiracetam can be used as first-line broad spectrum AED in children which is well tolerated and shown good seizure control.

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