**Abstract**— Epilepsy is a chronic disease occurring in approximately 1.0% of the world’s population. About 30% of the epileptic patients treated with available antiepileptic drugs (AEDs) continue to have seizures and are considered therapy-resistant or refractory patients. The ultimate goal for the use of AEDs is complete cessation of seizures without side effects. Because of a narrow therapeutic index of AEDs, a complete understanding of its clinical pharmacokinetics is essential for understanding of the pharmacodynamics of these drugs. These drug concentrations in biological fluids serve as surrogate markers and can be used to guide or target drug dosing. Because early studies demonstrated clinical and/or electroencephalographic correlations with serum concentrations of several AEDs, it has been almost 50 years since clinicians started using plasma concentrations of AEDs to optimize pharmacotherapy in patients with epilepsy. Therefore, validated analytical method for concentrations of AEDs in biological fluids is a necessity in order to explore pharmacokinetics, bioequivalence and TDM in various clinical situations. Levetiracetam is a new antiepileptic drug prescribed for the treatment of patients with refractory partial seizures with or without secondary generalization as well as for the treatment of juvenile myoclonic epilepsy. A wide variability in concentration-response relationships is expected in individual patients. Thus, the levetiracetam plasma concentration measurement could be used to help clinicians detect severe intoxication or therapy failure as well as to verify compliance. We applied homogenic enzymatic immunoanalysis for determination of levetiracetam plasma or serum levels in both paediatric and adult neurology patients on maintenance doses of oral drug. We have also compared concentrations measured by high performance liquid chromatography (HPLC) method with ultraviolet detection as a reference measuring parallely in an indipendent extramural laboratory with concentrations measured using our method. We found that there is excellent correlation as already published by some authors used other methods including HPLC and UPLC with tandem weight spectrometry. Potentially toxic level is considered as any levels > 37 µmol/l, but great concern should be given for existing inter and intra individual variability.

**Keywords**— ADEs Blood level Determination Methods, EMIT versus HPLC, TDM, Levetiracetam

**I. INTRODUCTION**

Epilepsy is a common neurological disorder in both paediatric and adult population affecting up to one percent of children for which the mainstay of treatment is anticonvulsant medication. Despite the frequent use of anticonvulsant drugs, remarkably little is known about the safety and efficacy of most of these medications in the paediatric epilepsy population. Many drugs have been used in the past to relieve symptoms by inducing changes in permeability to specific ions thus to stabilize membranes and interfering with release of neurotransmitters or other mechanisms. Although there is continuous emergence of new agents, pharmacoresistant symptom and adverse reactions including drug-drug interactions continue to challenge. Hardly preventable adverse effects (gastrointestinal, mental, or behavioral, neurologic or less commonly cutaneous, haematologic hepatic) by some agents may lead to reduction of quality of life, whereas some agents are known teratogens. Despite all developments in the field, no drug is considered as causal therapy for epilepsy to date. Thus the aim of antiepileptic drug prescription after management of emergency situation like stutus epileptici is prophylactic rather than therapeutic. Of 34 anticonvulsants currently approved for use by the US Food and Drug Administration (FDA), only 13 have been approved for use in children being among others since 2012 levetiracetam as an adjunctive treatment for partial onset seizures in infants and children from one month of age. Cormier and Chu concluded that the current data leading to the approval of levetiracetam for use in infants and children with partial onset seizures is encouraging, although more work needs to be done before definitive conclusions can be drawn about the efficacy of levetiracetam across different paediatric age groups[1]. The purpose of this
The company ARK Diagnostics produces the set for analysis of levetiracetam concentration in serum or plasma. The levetiracetam assay is an enzyme multiplied immunoassay technique (EMIT). It’s based on competition between levetiracetam in the specimen and levetiracetam labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding sites of antibodies (rabbit polyclonal antibodies). If the labeled levetiracetam molecules bind to antibodies, enzyme activity is decreased. The activity of G6PDH grows with increasing concentration of the drug from specimen. This enzyme catalyzes conversion of nicotinamide adenine dinucleotid (NAD) to NADH whose concentration is measured spectrophotometrically as a rate of change in absorbance. Samples have been analysed by HPLC-UV and EMIT (ARK Diagnostics) and the results were compared.

### III. Results

Sixteen samples were measured by EMIT method and within the same time have been also simultaneously determined by HPLC method. Thus, concentrations from all sixteen samples were valid for comparison. There is very good correlation between EMIT and HPLC-UV detection method. Pearson correlation coefficient 0.8265 means strong correlation dependence at 0.05 level of significance. There is also interindividual difference, although the dosing schedules were the same for patients under treatment (Fig. 1).
Table 2. Demonstrates correlation between values measured by compared methods also illustrated Fig. 2. (correlation graph) below.

IV. DISCUSSION

Epilepsy is a chronic disease occurring in approximately 1.0% of the world's population. About 30% of the epileptic patients treated with available antiepileptic drugs (AEDs) continue to have seizures and are considered therapy-resistant or refractory patients. The ultimate goal for the use of AEDs is complete cessation of seizures without side effects. Because of a narrow therapeutic index of AEDs, a complete understanding of its clinical pharmacokinetics is essential for understanding of the pharmacodynamics of these drugs. These drug concentrations in biological fluids serve as surrogate markers and can be used to guide or target drug dosing. Because early studies demonstrated clinical and/or electroencephalographic correlations with serum concentrations of several AEDs, it has been about half a century since clinicians started using plasma concentrations of AEDs to optimize pharmacotherapy in patients with epilepsy. In 1994 it was reported that the cost of uncontrolled epilepsy in the UK was £4167 per patient per year. That of controlled epilepsy was found to be £1630 [2]. If complete seizure control could reduce the costs of managing patients with epilepsy in a significant number of patients, the gains would be appreciable. TDM can help to improve seizure control in numerous ways including:

- identification of therapeutic failure due to underdosage,
- identification of therapeutic failure in the presence of 'optimal' dosage suggesting that a different AED should be tried,
- detection of non-compliance with prescribed therapy, which may be responsible for unnecessary and avoidable therapeutic failure,
- identification of the uncommon situation in which over-dosage causes increased seizures,
- detection of pharmacokinetic interactions which may compromise the adequacy of the therapy.

Nowadays, therapeutic drug monitoring (TDM) is widely accepted as a method to improve the effectiveness and safety of the first generation of AEDs and to identify an individual's optimum concentration and to individualize drug therapy [3]. Validated analytical methods for determining concentrations of AEDs in biological fluids is a necessity tool both in order to explore pharmacokinetics, bioequivalence studies and therapeutic drug monitoring (TDM). There are abundant published articles on the analysis of specific AEDs by a wide variety of analytical methods in biological samples. [4]. A new generation of antiepileptic drugs (AEDs) has reached the market in recent years with ten new compounds: felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, tiagabine, topiramate, vigabatrin and zonisamide. The newer AEDs in general have more predictable pharmacokinetics than older AEDs such as phenytoin, carbamazepine and valproic acid (valproate sodium), which have a pronounced inter-individual variability in their pharmacokinetics and a narrow therapeutic range. For these older drugs it has been common practice to adjust the dosage to achieve a serum drug concentration within a predefined 'therapeutic range', representing an interval where most patients are expected to show an optimal response. However, such ranges must be interpreted with caution, since many patients are optimally treated when they have serum concentrations below or above the suggested range. It is often said that there is less need for therapeutic drug monitoring (TDM) with the newer AEDs, although this is partially based on the lack of documented correlation between serum concentration and drug effects. Nevertheless, TDM may be useful despite the shortcomings of existing therapeutic ranges, by utilisation of the concept of 'individual reference concentrations' based on intra-individual comparisons of drug serum concentrations. With this concept, TDM may be indicated regardless of the existence or lack of a well-defined therapeutic range. From the ten newer AEDs, all have different pharmacological properties, and therefore, the usefulness of TDM for these drugs has to be assessed individually. For vigabatrin, a clear relationship between drug concentration and clinical effect cannot be expected because of its unique mode of action. Therefore, TDM of vigabatrin is mainly to check compliance. The mode of action of the other new AEDs would not preclude the applicability of TDM. For the prodrug oxcarbazepine, TDM is also useful, since the active metabolite carbamazepine is measured. For drugs that are eliminated renally completely unchanged (gabapentin, pregabalin and vigabatrin) or
mainly unchanged (levetiracetam and topiramate), the pharmacokinetic variability is less pronounced and more predictable. However, the dose-dependent absorption of gabapentin increases its pharmacokinetic variability. Drug interactions can affect topiramate concentrations markedly, and individual factors such as age, pregnancy and renal function will contribute to the pharmacokinetic variability of all renally eliminated AEDs. For those of the newer AEDs that are metabolised (felbamate, lamotrigine, oxcarbazepine, tiagabine and zonisamide), pharmacokinetic variability is just as relevant as for many of the older AEDs. Therefore, TDM is likely to be useful in many clinical settings for the newer AEDs. The purpose of the present review is to discuss individually the potential value of TDM of these newer AEDs, with emphasis on pharmacokinetic variability. [5]. The rationale for the determination of AEDs and their metabolites in body fluids and tissues arises from different fields of investigations and clinical situations. Either drug or metabolites levels are required for regular monitoring of therapeutic drug levels, for adverse drug reactions, for drug–drug interaction studies, for issues of toxicity concern, for pharmacokinetic, pharmacokinetic/pharmacodynamic and bioequivalence studies. AEDs are often used in polypharmacy including up to three different AEDs, each of them having several own metabolites [6]. TDM is more important for drugs with a narrow therapeutic range, where a correlation has been established between drug concentration and its therapeutic and toxic effects. Although reasonably well-defined target ranges in serum concentrations have been determined for most of the established AEDs [7, 8], it should be remembered that these ranges only became established after the development and general availability of sensitive and reliable analytical methods. Thus, even though phenobarbital and phenytoin became use for clinical application in the early 1900s, after the development of analytical methods in the 1960s, it was only since the early 1970s that target ranges were identified [9, 10]. Since then, monitoring AEDs such as carbamazepine, vaproate and ethosuximide has also become widely accepted in clinical practice [11]. Although the target ranges have been defined for some of the AEDs, the true therapeutic range is defined for a given patient as the concentration that prevents occurrence of epileptic episodes without causing side effects. Since 1989, several AEDs have been have been approved for clinical use, and because their regulatory trials were not serum concentration controlled or designed to investigate the relationship drug concentration and effect, the value of monitoring these drugs is presently controversial. However, some of the newer AEDs have pharmacological properties suggesting that their optimal use may be facilitated by use of TDM, and this has been the subject of recent valuable debate [12, 13]. The AEDs have been measured by a wide variety of analytical methods in serum, blood, saliva, urine and tissue. For the classic AEDs (carbamazepine, ethosuximide, phenobarbital, vaproate) and some of the new AEDs (felbamate, topiramate, zonisamide etc), automated enzyme-multiplied immunoassay technique (EMIT) and fluorescence polarization immunoassays (FPIA) are available and allow rapid and accurate determination of concentrations in biological fluids, usually serum or plasma. For other AEDs laboratories rely on chromatographic methods; gas-chromatography (GC) and high-performance liquid chromatography (HPLC) with a various detection methods, which are more labor-intensive and relatively more expensive. A number of simultaneous chromatographic assays for AEDs have been developed in the past. The early initial simultaneous AED assays, from 1970s and 1980s, concentrated on separating the older AEDs such as ethosuximide, primidone, carbamazepine, carbamazepine-10,11-epoxide, phenytoin, and phenobarbital ([14-18] Many subsequent assays separated the same compounds with the inclusion or removal of one or more additional drugs or metabolites such as ethylpenacemide [16], 5-parahydroxyphenyl-5-phenylhydatoin, N-desmethyldesuximide [17], and lamotrigine and phenyl-2-thyl-malonamide [19]. Another inclusive assay separated ethosuximide, primidone, carbamazepine, phenytoin, Phenobarbital, carbamazepine metabolites, phenobarbital metabolite and felbamate [20]. All of these assays employ ultraviolet (UV) detection, thereby increasing the risk of metabolite or metrix interferences. Assay developed over past 15 years have focused more separating newer AEDs. There are also new technological advance in the use of capillary electrophoresis (CE) for TDM. Like other chromatographic methods, CE allows simultaneous measurement of several AEDs and can provide automation of procedures, low cost, and rapid speed with high specificity [21, 22]. The number of articles with the analytical assay of AEDs in various biological matrices is increasing in accordance with growing interest in the situations with pharmacokinetic, TDM and bioequivalence studies of clinical and research fields. The present review was to focus to current technologies applied to the analysis of AEDs in biological media for monitoring individual AEDs or simultaneous monitoring of AEDs in recent years. Levetiracetam is among the latest AEDs to be licensed for clinical use indicated for adjunctive treatment of intractable partial seizures that do not respond to other AEDs. Its mechanism of action is not clearly defined, [23], whereas its pharmacokinetics such as rapidly absorption from the gastrointestinal tract, non-significant protein binding and exhibition of linear pharmacokinetics.is well known It is minimally metabolised and excreted essentially unchanged via the kidneys. Levetiracetam has no propensity to interact with other AEDs [24].There is little published information on levetiracetam blood levels in patients with epilepsy. Nevertheless, audit of the clinical trials data for levetiracetam suggests a target range of 35 - 110 μm/L, while blood levels can be measured by HPLC with ultraviolet detection [25]. Levetiracetam is relatively a new antiepileptic drug prescribed for the treatment of patients with refractory partial seizures with or without secondary generalization as well as for the treatment of juvenile myoclonic epilepsy. A rapid and specific method by high-performance liquid chromatography diode array detection was developed to measure the concentration of levetiracetam in human plasma. A wide variability in concentration-response relationships was observed in patients.
Nevertheless, the levetiracetam plasma concentration could be used to help clinicians detect severe intoxication or to verify compliance by repeating the measurement in patients [26]. An isocratic high performance liquid chromatographic micromethod, Ratnaraj et al. reported that no interference from commonly prescribed antiepileptic drugs (carbamazepine and its metabolite carbamazepine epoxide, ethosuximide, gabapentin, lamotrigine, phenobarbital, phenytoin, primidone, valproic acid, and vigabatrin) was observed, and thus the method can be used to monitor levetiracetam in patients on polytherapy antiepileptic drug regimens. [27]. In a study to evaluate the efficacy and tolerability of levetiracetam (LEV) as add-on therapy in children with refractory epilepsies and to determine the value of LEV blood level monitoring in this population, Giroux et al. published that the most frequently observed adverse effects were drowsiness, behavioral difficulties, increase in seizure frequency and headaches. The majority (60.5%) of the responders received doses between 10 and 50mg/kg/day and had a plasma concentration between 5 and 40mcg/ml, however, no clear correlation between drug plasma concentration and efficacy has been found. [28].

According to the study to evaluate the efficacy and tolerability of Levitiracetam in a large pediatric cohort with drug-resistant epilepsy from a prospective multicenter observational study it has been reported that Levitiracetam is a well-tolerated new antiepileptic drug that may effectively improve seizure control as an add-on drug in resistant epilepsy in childhood with good tolerability, however, neurologically handicapped children appear at increased risk for reversible neurocognitive side effects and have a poorer treatment response. [29] Levitiracetam is among the most recently licensed antiepileptic drug (AED) for adjunctive therapy of partial seizures. Its mechanism of action is uncertain but it exhibits a unique profile of anticonvulsant activity in models of chronic epilepsy. [30].

Most studies suggest that levitiracetam is effective against partial and generalized epilepsy. In resistant partial epilepsy, the percentage of responders reaches 64%, with 8 to 23% seizure free. Levitiracetam is used to treat symptomatic and idiopathic epilepsies. The drug has also proven effective against photosensitivity and epileptic and nonepileptic myoclonus. The most frequent side effects involve the behavioral sphere and manifest mostly in patients with a history of behavioral problems. In some patients, levitiracetam increases the number of seizures, but this adverse reaction can be partially avoided with slow titration. Doses for children should be 130 to 140% of those advised for adults. [31]. Treatment of seizures in pediatric patients is complicated by the fact that the etiology of the disorder and the pharmacokinetics, efficacy, and safety of antiepileptic drugs may differ from that in adults. With few controlled clinical trials of AEDs in children, the selection of agents to treat paediatric patients must be made on the basis of information from small uncontrolled studies or the extrapolation of clinical trial results in adults. Data from a large number of children with a wide range of seizure disorders who were treated in small-scale prospective studies, or whose records were retrospectively evaluated, indicate that levitiracetam reduces the frequency of seizures in pediatric patients. Available data also indicate that levetiracetam is well tolerated in pediatric patients, with a safety profile similar to that in adults, a low potential for behavioral disturbances, and no reported idiosyncratic adverse reactions. As with other AEDs, children metabolize and clear levetiracetam more rapidly than adults, and somewhat higher doses (based on body weight) are needed to achieve desired plasma concentrations. Several ongoing studies will provide further information on the pharmacokinetics, efficacy, and safety of levetiracetam in this patient population. [32]. Some study results support the use of a weight-based LEV dosing regimen and provide a basis for a recommended pediatric dosage regimen, but the relationship between LEV plasma concentrations and clinical effect has not been evaluated fully and could differ between adults and children and Clinical studies should be able to validate these dosing recommendations. [33]. Some significant covariates for pharmacokinetics of levetiracetam were identified: (a) age on the absorption rate constant; (b) bodyweight, dose, clearance, and concomitant enzyme-inducing AED on plasma oral clearance (CL/F); and (c) bodyweight on the apparent volume of distribution after oral administration (V(d)/F). The main explanatory covariates were age on absorption rate constant, bodyweight on CL/F and V(d)/F, and enzyme-inducing AED on CL/F, of which bodyweight was the most influential covariate. However, the most influential covariate of levetiracetam pharmacokinetics in children is bodyweight. [34]. Both serum and CSF levetiracetam concentrations rose essentially linearly and dose-dependently, suggesting that transport across the blood-brain barrier is not rate limiting over the levetiracetam concentration range observed. However, while apparent elimination half-life (1/2) values for both serum and CSF were dose-independent (mean value range 1.8-2.8 and 4.4-4.9 h, respectively), 1/2 values for CSF were significantly larger. As the serum free/total serum levetiracetam concentration ratio (free fraction) was 1.01+/-0.02 (mean+/-S.E.M.), it can be concluded that levetiracetam is not protein bound. [35]. Levetiracetam is completely and rapidly (Tmax, 1 h) absorbed after oral ingestion with bioavailability of 100% [36, 37]. Although drug-food interactions do not show on the extent of absorption, rate of absorption is slowed in the presence of food. Administration of a crushed LVT tablet together with 120 ml of an enteral nutrition formula has been associated with a mean 27% decrease in peak LVT concentration, but the effect was not statistically significant. [38]. Levetiracetam shows linear pharmacokinetic and renal elimination with approximately 66% of a dose eliminated unchanged and 27% as inactive metabolite [39]. The relationship between LVT serum concentrations and clinical effect has not been ascertained, and consequently the value of serum concentrations measurements is not established. Because of its favorable therapeutic index, low plasma protein binding and minimal side-effect profile, routine monitoring of LVT serum concentrations appears to be unnecessary for safe use of the drug, and dosing can be readily guided by the therapeutic response [40, 41]. Nevertheless, its use in ascertaining
compliance and managing patients that are overdosed would be helpful. Because LVT has a relatively short half-life, sampling time in relation to dose ingestion is important for the interpretation of the drug concentration. Ideally samples for LVT measurements should be drawn before the morning dose. Because LVT can undergo in vitro hydrolysis, it is important to separate whole blood from serum as soon as possible so as to avoid LVT hydrolysis that would result in spuriously lower concentrations being measured [42]. Although AED monitoring in saliva may some clinical applicability, it has not yet come into routine use, but, a significant positive correlation exists between LVT saliva and serum concentrations, LVT like other AEDs, can be measures in saliva as an alternative to blood-based assays for minotoring the LVT therapy [43, 44]. Numerous chromatographic methods have been reported for the quantification of LVT in biological fluids. Different HPLC methods for the determination of LVT in human plasma have been reported coupled with UV [45, 46], or diode array detection [47, 48]. mostly after sample pretreatment by expensive solid-phase extraction or time-consuming liquid-liquid extraction procedures [46, 48]. The availability of simple, accurate and inexpensive analytical assays is crucial for the successful use of TDM in clinical practice. LVT spiked plasma sample preparation by different kinds of deproteinization before HPLC-diode array detection was first explored by Pucci et al[47], and subsequently applied to patient samples analysis by HPLC-UV [49, 45, 50]. Otherwise, these involve GC-NPD [51]. and GC-MS [52]. Most of the reported methods lack selectivity, sensitivity, and reliability. They encounter also problems particularly tedious and time-consuming sample preparation as well as high sample volume. Recently, LC-tandem MS is considered a gold standard to utilize in analysis of drugs in biological fluids. The high sample throughput, selectivity and sensitivity for analytes of interest may increase the applicability of tandem MS in clinical chemistry as well as clinical studies [53-55].

V. CONCLUSIONS

We found that there is excellent correlation as already published by some authors used other methods including HPLC and UPLC with tandem weight spectrometry. Potentially toxic level is considered as any levels > 37 umol/l, but great concern should be given for existing inter and intra individual variability. Our method is suitable for routins levetiracetam plasma level determination in the process of therapeutic drug monitoring.

References


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