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Short communication

Correlation of saliva and serum free valproic acid concentrations in persons with epilepsy

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ABSTRACT

Purpose: Therapeutic drug monitoring (TDM) of antiepileptic drugs (AEDs) in serum is frequently used in clinical settings however saliva could be an alternative to measure free concentration of drugs. In the present study, we observed the possible correlation of VPA concentration between serum and saliva in persons with epilepsy (PWE).

Methods: A total of 59 paired serum and saliva samples were assayed from 65 consecutive PWE (51 males and 14 females; age range 9–65 years). Patients were subjected to either VPA monotherapy or its combination with other AEDs for at least three months. Steady state trough concentration of unbound VPA drug was quantified using HPLC. The correlation between serum and saliva free VPA concentration was evaluated.

Results: Out of 65 patients, 27 were on monotherapy of VPA and 38 were on VPA with other antiepileptic drugs. Saliva VPA concentration significantly correlated with serum free VPA concentration ($p < 0.05$). Poor correlation was observed between serum and saliva VPA concentration with the daily dose ($p > 0.05$) respectively.

Conclusions: Our study reveals that serum and saliva VPA concentrations are significantly associated in PWE. These associations may facilitate monitoring and evaluation of VPA levels non-invasively for PWE.

1. Introduction

Valproic acid (2-propyl pentanoic acid, VPA) is a broad spectrum AED and is frequently prescribed by physicians to control various forms of seizures and syndromes. It is also indicated for the treatment of diseases like schizophrenia, bipolar disorder, depression, neuropathic pain and prophylactic treatment of migraine.[1] TDM of VPA plays an important role in understanding its efficacy as well as toxicity. As clinical effects of therapy are more closely related to the levels of drug than dose, TDM may be helpful to optimize the dose in order to have enhanced efficacy and to minimize toxicity.[2,3] In TDM, blood samples are commonly used for monitoring the concentrations of various AEDs in a routine practice. As of now, there in evidence supporting saliva being used as an alternative to serum for monitoring the free concentration of AEDs and few studies have shown a significant association between serum and saliva concentration in PWE.[3,4] In different clinical settings, several pharmacokinetic and pharmacodynamic studies were reported which showed either strong or weak association for the VPA levels in both serum and saliva. However these associations were affected by either variability of pH, interpatient variability, precision of different assays, drug–drug interactions or genetic variations in diverse ethnic population.[2,3,8] In some reports, it has been shown that a significant correlation of saliva levels with serum levels of VPA is there using different approaches,[5,7] whereas others have observed no association.[6,8,9] These inconsistent reports give an opportunity to investigate this aspect in other populations. In the Indian population, serum samples for VPA estimation were observed however to the best of our knowledge this is a first report in Indian ethnicity where we used saliva for VPA estimation in PWE. We studied the association of free VPA concentrations to ascertain the correlation between serum and saliva in PWE.

2. Materials and methods

2.1. Ethical approval and study subjects

All PWE were prospectively recruited from 2010 to 2012 at the out-patient epilepsy clinic, Department of Neurology, All India

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Institute of Medical Sciences (AIIMS), New Delhi, India. This study was approved by Institute Ethics Committee and written informed consent was obtained from all subjects or guardians prior to sample collection. PWE, who were taking either VPA alone or in combination with other AEDs for a minimum of 3 months; able to produce saliva and age ≤65 years were included in our study. PWE who were either unable to give consent or had history of illness like stroke, tuberculosis, diabetes, endocrinal disorders, AIDS etc. or had adverse effect of AEDs were excluded. Diagnosis of epilepsy was made according to International League Against Epilepsy (ILAE) guidelines; 2010. Clinical and demographic details were recorded during recruitment.

2.2. Sample collection and determination of free VPA concentration by HPLC

Venous blood samples (3 mL) were collected at trough level (just before the next dose) in plain vials (BD Vaccutainer tube, BD Bioscience, USA), at the same time, saliva samples were also collected by placing commercially available salivette cotton swab with citric acid (Sarstedt, Nümbrecht, Germany). Blood and saliva samples were centrifuged within 2 h at 3000 rpm for 10 min at 4 °C. Clear supernatant of serum and saliva fractions were stored at −80 °C until further analysis. The free concentration of VPA in serum and saliva was quantified by HPLC System (Agilent 1200 series, Agilent Technologies, USA) using Chromolith RP, 18e (100 mm × 4.6 mm) packed column (Merck, Germany). Further analysis was carried out as described method by Kishore et al.[10] with slightl alterations (flow rate 1 mL/min, injection volume 25 μL, column oven temperature 35 °C, run time 10 min and wave length 210 nm). The standard curve of VPA for saliva and serum was plotted in the different concentrations range (5–100 μg/mL). Less than 5% intra and inter day variability for VPA was observed.

2.3. Statistical analysis

All data were analyzed using STATA [v. 11.0 (Statacorp, College Station, TX, USA)] and summarized as mean ± SD. The Pearson correlation coefficient was used when data had a normal distribution. Otherwise, the spearman rank-correlation coefficient was performed to elucidate the correlation between serum and saliva VPA concentration. The $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Demographic and clinical details

A total of 65 PWE were recruited with the age range between 9 and 62 years (22.70 ± 9.96, mean ± SD) and their body weight ranged 26.0–98.4 kg (53.55 ± 12.92, mean ± SD). The percentage of male (78.5%) was higher than female (21.5%). The mean ± SD for duration of epilepsy, age at onset of seizures, frequency of seizures is presented in (years) and duration of treatment in (months) were 7.68 ± 6.93, 15.09 ± 9.92, 76.12 ± 260.47 and 52.92 ± 56.01 respectively. Patients with generalized seizures (61.5%) were higher as compared to focal seizures (38.5%) and seizures control was achieved in 18 (27.7%) patients with one or two AEDs regimen whereas 47 (72.3%) patients had drug refractory epilepsy (DRE) with more than two drugs. Out of 65 PWE, higher percentage of patients (58.5%) were taking VPA in combination with other AEDs, i.e. phenytoin, carbamazepine, phenobarbital, levetiracetam, lamotrigine and clobazam etc. at a daily dose of (996.05 ± 418.23 mg/day mean ± SD) as compared to VPA (41.5%) alone (807.40 ± 281.37 mg/day mean ± SD). Drug doses were adjusted with their body weight (53.55 ± 12.92 kg; range, 26.0–98.4 kg). In 65 patients, 41.5% of PWE were on monotherapy, 40% of PWE on duotherapy whereas 18% of PWE on polytherapy. As shown in Fig. 1, the levels of VPA in mono-, and poly-therapy were not found statistically significant ($p > 0.05$) in free serum and saliva whereas in duotherapy PWE it showed a statistically significant association ($p < 0.05$). Implying that the interaction of two AEDs may alter the level of drugs in PWE. Interestingly, with increase in the number of drugs in poly-therapy increased, it remains unchanged as in monotherapy.

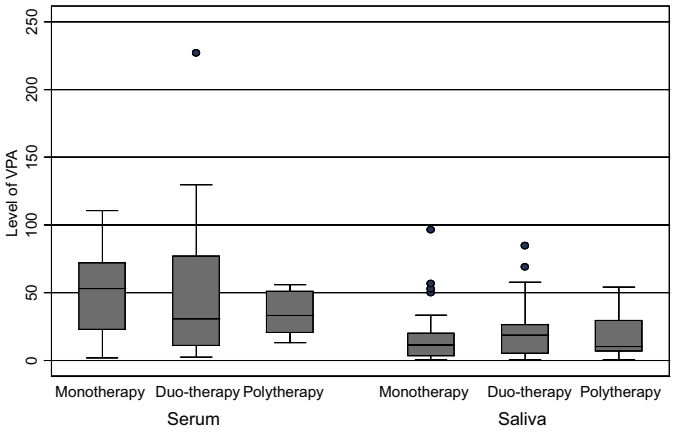

Fig. 1. Comparative graphical presentation for mono-, duo- and poly-therapy of PWE with VPA level in serum and saliva.

3.2. Correlation between saliva and serum unbound VPA concentration

Out of 65 PWE, paired samples (saliva and serum) were analyzed in 59 subjects. The VPA levels of serum in 6 patients were below the limit of quantification. However, mean ± SD (11.5 ± 8.5 μg/mL) of VPA levels of saliva in these subjects were observed. These patients were excluded for correlation analysis. The mean ± SD of VPA concentration in free serum and saliva were 47.35 ± 39.02 μg/mL and 19.02 ± 19.43 μg/mL respectively. The mean ratio of saliva to serum free concentration of VPA was 0.68 ± 1.29%. Free serum VPA concentrations significantly correlated with that of Saliva VPA concentrations ($r = 36$; $p < 0.004$) while determination of correlation coefficient (r^2), slope, and confidence interval (CI) were 0.13, 0.73 and 0.23–1.22 respectively. As shown in Fig. 2A, we observed a linear relationship between the saliva and serum free concentration of VPA. However, poor correlation coefficient was observed between serum and saliva VPA concentration with daily dose ($r^2 = 0.01$ and $r^2 = 0.02$) respectively (Fig. 2B – left and right). These associations were not statistically significant ($p > 0.05$) and showed non-linear relation in PWE. As expected, we found that drug dose significantly correlated with age and weight ($r = 0.39$ and $p < 0.005$), ($r = 0.40$ and $p < 0.0001$).

4. Discussion

TDM is highly useful in determination of drug levels and identification of therapeutic failure either due to under or optimal dose or toxicity due to an inter individual variability. Monitoring of VPA using saliva as compared to serum is an essential approach for the determination of free drug concentrations.[4] Through simple diffusion, free drug transports across the biological membrane and reaches the effector site according to the concentration gradient.[2,3] In our study by comparing the VPA estimation in saliva and serum, we observed that 64.3% of