

Phenytoin concentration in people with epilepsy: a comparative study in serum and saliva

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Abstract

In clinical practice, therapeutic drug monitoring (TDM) makes it possible to measure the concentration of drugs in serum or saliva, the purpose of which is to reduce adverse effects and optimize pharmacological therapy. The objective was to determine the concentrations of Phenytoin in saliva and serum of people with epilepsy. Cross-sectional, descriptive study with dynamic recruitment of 30 people with epilepsy (n = 30; 17 men, 56.7% and 13 women, 43.3%; mean age 33.9 ± 11.83 years). Serum and saliva samples were collected at trough levels from patients, who were under phenytoin treatment for at least three months. Drug levels were assessed by the Cloned Donor Enzyme Immunoassay method. Phenytoin levels were found in saliva between 0.01 to 3.56 mg/L and in serum between 0.09 to 36.60 mg/L. Pearson's analysis showed an association between the estimated serum and saliva phenytoin concentrations (R² 0.7026; 95% CI 0.685-0.921), with a significant statistical correlation (p < 0.05). The Bland-Altman test broke concordance, the difference between the two saliva/serum methods is within 95% confidence. It is concluded that there is an association and concordance between the concentrations of phenytoin in serum and saliva, therefore, this technique can be useful in the clinical monitoring of phenytoin.

Keywords

Phenytoin, saliva method, serum, phenytoin monitoring, therapeutic range

Introduction

Epilepsy is a chronic neurological disorder characterized by self-limited seizures with a high probability of recurrence within the next 10 years (Fisher et al. 2017; Alvarado et al. 2020). Seizures occur due to abnormal depolarization of neurons, manifesting clinically with sudden and transient episodes of motor, sensory and autonomic origin, which are observed as prolonged and severe muscle contractions, loss of sphincter control, loss of consciousness, altered senses and mood (Dwivedi et al. 2016). Precision medicine is applied in the treatment of epilepsy, which is based mainly on ethnicity, pharmacogenetic profile, and the plasma level of the drug (Aronson and Rehm 2015; Antman and Loscalzo 2016; Ohno et al. 2018; Alvarado et al. 2019).

Phenytoin is an antiepileptic drug (AED) derived from hydantoin, chemically named 5,5-diphenylimidazolidine-2,4-dione (PHT), used in status epilepticus (Guk et al. 2019), tonic-clonic seizures, and complex partial seizures (Dwivedi et al. 2016; Selioutski et al. 2017; Alqahtani et al. 2019); this drug is absorbed in the gastrointestinal mucosa, generating a bioavailability of 80%, which is reduced by drug-drug, drug-nutrient interactions and malabsorption syndrome (Milosheska et al. 2015; Darlic and Amudio 2016; Alvarado et al. 2020). Phenytoin has a narrow therapeutic index, in this sense, a small variation in the dose can cause the minimum effective plasma concentration (C_{ME} : 10 mg/L) not to be reached, increasing the risk of seizures; or exceeding the minimum toxic concentration (C_{MT} : 20 mg/L), predisposing to adverse effects and toxicity (Thaker et al. 2017); another factor that influences the plasma levels of phenytoin are the genes that express the isoenzymes CYP2C9 and CYP2C19, which metabolize phenytoin into 5-(p-hydroxyphenyl)-5-phenylhydantoin (p-HPPH), an inactive metabolite (Fig. 1A) (Alvarado et al. 2020); therefore, patients who are poor metabolizers with *CYP2C9**2 and *CYP2C9**3 allelic variants are prone to drug-induced toxicity (Fig. 1B) (Alvarado et al. 2019; Bartra et al. 2021).

In clinical practice, therapeutic drug monitoring (TDM) makes it possible to measure the concentration of drugs in serum or saliva (Nwobodo 2014; Hutchinson

et al. 2018). Due to the narrow therapeutic index, non-linear pharmacokinetics, variable absorption and metabolism by polymorphic enzymes (Kaewdoung et al. 2015; Javadi et al. 2018; Shaikh et al. 2018), clinical monitoring of phenytoin by the saliva method is warranted (Carvalho et al. 2019). This method reflects the free fraction of the drug that reaches the brain; rapid, simple and non-invasive, it prevents distress and guarantees patient safety, being an alternative for the clinical monitoring of phenytoin in children and older adults, at the same time it is economical for its use in Latin American countries.

The objective was to determine the concentrations of phenytoin in saliva and serum of people with epilepsy, to know if there is a correlation and concordance of the salivary method, and to be used as an estimator of its serum levels during antiepileptic therapy.

Materials and methods

Study design and population

Descriptive, cross-sectional, non-randomized study and with dynamic recruitment (Dwivedi et al. 2016; Alvarado et al. 2022) was carried out in the outpatient clinic of the Neurology Service of a hospital in Mérida, Venezuela, from March 2019 to February 2022. Neurologists diagnosed the patients according to the guidelines of the International League Against Epilepsy (ILAE) (Berg et al. 2010).

Biological sample

A single blood and saliva sample was obtained from each patient who attended for control and as part of routine medical practice. Saliva was collected naturally and without additional stimulation, which was done 10 hours after the last dose of the drug; Said saliva was collected in previously coded glass tubes. Blood collection was performed 10 h after the last dose of the previous day (Mennickent et al. 2007; Alvarado et al. 2020; Alvarado et al. 2022). These samples were stored refrigerated at -21 °C until analysis.

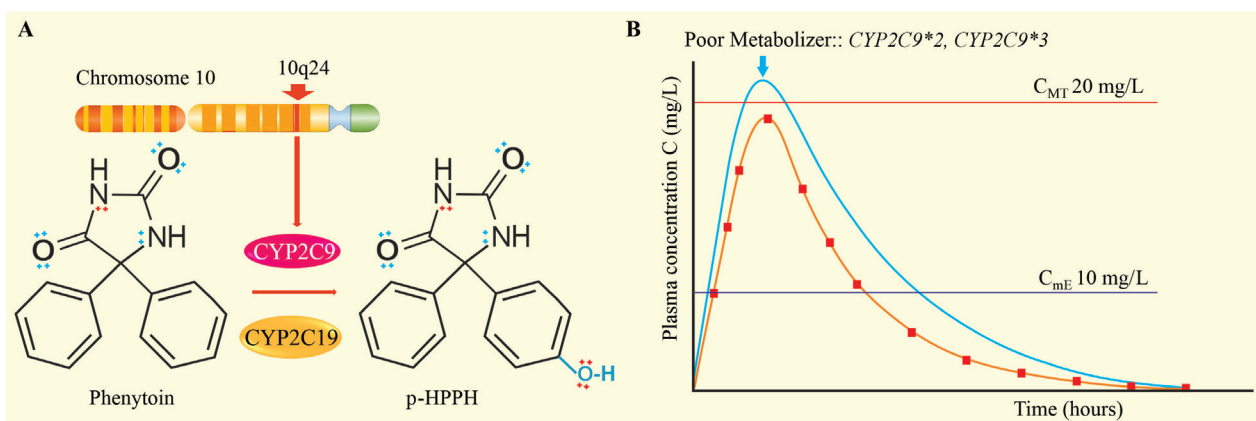


Figure 1. Metabolism and plasma level of phenytoin in people with a slow metabolic phenotype. Figure made by the authors.

Selection criteria

Patients treated with doses of 100 mg of phenytoin (generic tablets) every 8 hours, and with no less than three months of medication were included. They were instructed to not consume other medications, comply with the dose and frequency of administration of the antiepileptic.

The patients who agreed to participate in the study signed the informed consent form before the extraction of the biological samples, they were immediately assigned a code to guarantee confidentiality and anonymity (Alvarado et al. 2019, 2022).

Phenytoin quantification

3 mL of venous blood was drawn into Vacutainer tubes, BD Bioscience, and saliva (1 mL) was collected into glass centrifuge tubes containing 20 mg of the anticoagulant sodium citrate (chelator of Ca^{++} ions) (Guk et al. 2019). For the quantification of the drug in serum and saliva, the samples were centrifuged within two hours of sampling at 8,000 rpm for 10 minutes. The clear supernatant was collected from the serum and saliva fractions and stored at $-21\text{ }^{\circ}\text{C}$ until analysis (Dwivedi et al. 2016; Alvarado et al. 2020). Then, 0.5 mL of each biological sample was measured, without requiring any special treatment, to determine total phenytoin in serum and its free form in saliva, by the CEDIA method (cloned donor enzyme immunoassay) in the Indiko equipment. Thermo Fisher Scientific (Waltham, Massachusetts, USA) (Mennickent, et al. 2007). In Fig. 2 schematically represents the selection of patients, and the taking of the biological sample.

Ethical aspects

The study was developed in strict compliance with ethical standards, and criteria of the Belmont Report, Declaration of Helsinki with the current revision. The Institutional Medical Board approved this study as a minimal risk investigation, for using blood samples and saliva from routine clinical practice, through certificate 003-JMI-2019. Each volunteer was assigned a code to guarantee confidentiality and anonymity.

Statistical analysis

Analysis of variance (ANOVA) and Pearson's correlation test was applied to evaluate the possible association between the concentration of phenytoin in serum and the concentration in saliva; and the Bland-Altman method to calculate the confidence intervals of the differences and estimate the precision of the result. A value of $p < 0.05$ was considered statistically significant.

Results

We enrolled 30 volunteer patients with generalized tonic-clonic seizures and simple or complex partial seizures [17 males (56.7%) and 13 females (43.3%)], who, at the time of taking the sample, consumed for three months, a dose of 100 mg of sodium phenytoin every 8 hours. It was observed in one patient that the minimum toxic concentration was exceeded (36.60 mg/L). The average ratio of PHT concentrations (saliva 1.10/serum 10.53) is 0.10 (Table 1).

Table 1. Demographic and clinical data of patients (n = 30).

Statistics	Age (years)	Weight (kg)	Dose (mg)	Saliva concentration (mg/L)	Serum concentration (mg/L)
Median	28.0	66.00	300	0.82	10.30
Minimum	19.0	50.00	100	0.01	0.09
Maximum	62.0	96.00	600	3.56	36.60
Mean	33.9	68.91	290	1.10	10.53
SD	11.95	11.32	95.95	0.795	6.744

SD standard deviation; n number of patient volunteers.

The concentration level (mg/L) of the patients was determined (Fig. 3A), and the dose (mg/day)-concentration (mg/L) relationship and the therapeutic range (Fig. 3B), in 60% of patients the concentration is within the therapeutic range. At ANOVA, the difference in means is significant ($p < 0.05$).

Fig. 4 shows the association result using Pearson's correlation coefficient. It was found that the results are statistically significant ($p < 0.05$) (Fig. 4A). Using the Bland-Altman method, the mean differences in saliva/serum concentrations were compared with 95% confidence intervals (CI) (95% CI) (Fig. 4B).

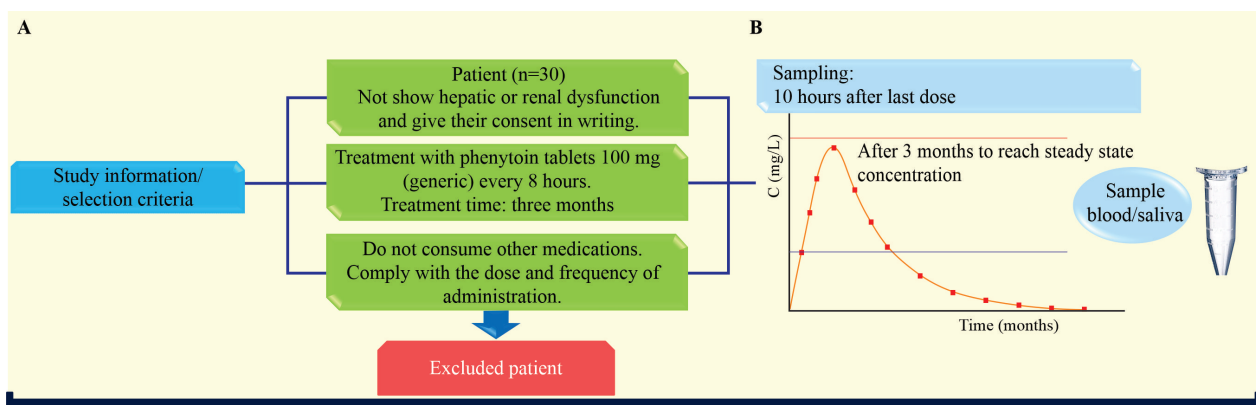


Figure 2. Representation of the selection criteria, moment, and sampling.

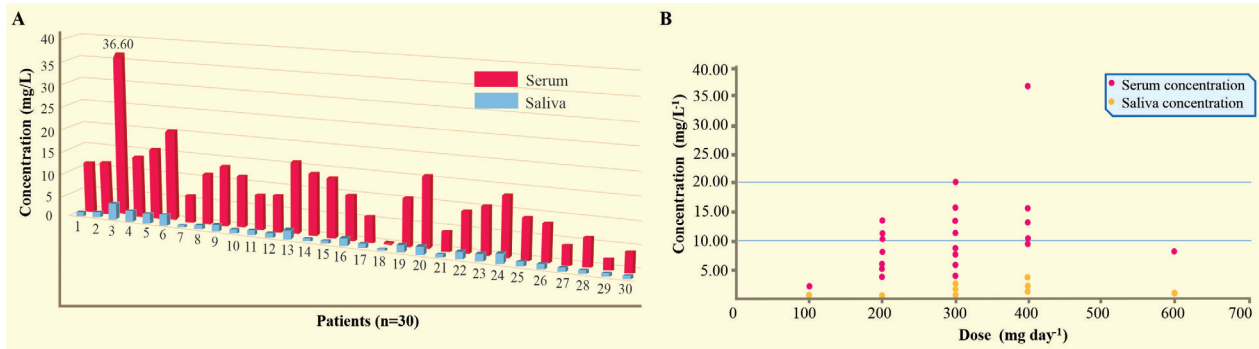


Figure 3. Concentration (mg/L) of biological samples in patients and dose-concentration relationship of phenytoin showing the therapeutic range.

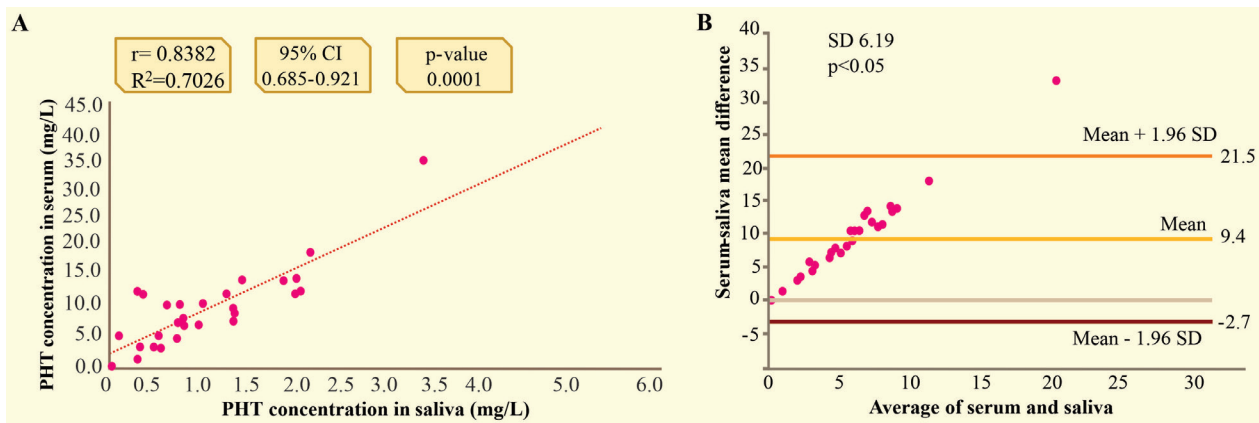


Figure 4. Dispersion diagram between phenytoin concentrations in serum and saliva (A) and Bland-Altman graph evaluating concordance between the reference method (serum) and the saliva method to estimate the concentration of phenytoin, in mg/L (B).

Discussion

It is known that phenytoin has a narrow therapeutic range, with a minimum effective concentration of 10 mg/L and a minimum toxic concentration of 20 mg/L. In 60% of the patients in the study, it is observed that the values of serum concentrations are within the values of the therapeutic range; with a serum mean of 10.53 ± 6.74 mg/L. Regarding the concentration of phenytoin found in saliva, this was 1.10 mg/L (SD 0.79), which corresponds to 10% of that observed in the serum. Concentration values in saliva have also been established, with the optimal level being 1.5 mg/L, below 0.78 mg/L is considered subtherapeutic, while a value above 5.4 mg/L is responsible for toxicity (Ibarra et al. 2010). For it, patients had to avoid the co-administration of two or more drugs, avoid enzyme inducers, administration with nutrients, preventing interactions between them (Alvarado et al. 2021a), at the same time, the metabolic genotype and phenotype of each patient should be evaluated, to avoid therapeutic failures or drug toxicity. However, a serum concentration of 36.60 mg/L was observed in one patient (3.33%), and the medical examination presented gingival hyperplasia, so it was decided to suspend the dose of the drug, and subsequently, the change in treatment was evaluated. In the case of the group of patients with subtherapeutic levels, it was suggested to adjust the dose based on the dose level index

(N/D), perform the Pharmacotherapeutic Follow-up for compliance with pharmacological therapy, and to detect possible adverse effects with the new dose. Our result is close to that reported by Mandal et al., who found 71.4% ($n = 15$) had plasma phenytoin levels within the therapeutic range, in one patient (4.8%) a level higher than therapeutic was observed. (Mandal et al. 2019). While Shaikh et al., found that 14.5% ($n = 7$) of patients had plasma phenytoin levels within the therapeutic range and 16.5% ($n = 8$) above the minimum toxic concentration (Shaikh et al. 2018).

ANOVA was applied to determine if there is any difference between the means of the concentrations in saliva and serum, observing a very small p-value ($p = 0.00000000273$) indicating that the result of the study is significant and reliable with 5% of error, and is clinically important. To analyze the relationship of the cumulative levels of phenytoin in serum/saliva, a scatter diagram was made for the entire study population, observing a linear relationship, with a positive association according to the Pearson test ($R^2 = 0.7026$), which indicates that as the concentration in serum increases, it also increases in saliva; and the 10% concentration in saliva is always maintained concerning the serum value. These results indicate a statistically significant association of the levels of phenytoin in both biological matrices, but clinically they would not be correlated by some levels of phenytoin in serum that

exceed the C_{MT} values, masking a possible overdose. Therefore, the Bland-Altman method was applied to find the limits of agreement of the saliva/serum methods, and it was observed that the difference between the two methods is within 95% confidence, whose values are around 0 (in which there is no difference) and within the upper bound (21.5), except one value that is outside the limits of agreement. Based on the association and concordance, it can be suggested that the saliva method could be an estimator of serum levels (Patsalos and Berry. 2013). Our findings are substantiated and relate to other previously published studies. In 2016, Dwivedi et al. observed a correlation coefficient range (r) of 0.65–0.99 in the antiepileptic drugs studied (Dwivedi et al. 2016). This variation may be due to the saliva collection methodology, nonlinear pharmacokinetics, and interindividual variability (McAuliffe et al. 1977). For their part, Hutchinson et al. demonstrated that the saliva method is suitable for monitoring the concentration of carbamazepine, ethosuximide, phenytoin, phenobarbital, and primidone (Hutchinson et al. 2018); achieving stable steady-state concentration of the drug in plasma ensures measurable and stable concentrations in saliva (Patsalos and Berry 2013; Hutchinson et al. 2018).

The limitations of the present investigation are in the declaration of the patients of having met the selection criteria, the sample size ($n = 30$) that was not calculated based on the number of patients with epilepsy who attend the Neurology Service, so alpha and beta errors are not reported. Other biases that can lead the confusion are the selection of the sample that was for convenience and not random, which should be considered to obtain a more robust statistical relationship in future studies; another limitation is having used the cloned donor enzyme immunoassay method (CEDIA), being necessary to carry

out future studies using the high-resolution chromatographic method to obtain values of greater sensitivity and improve the associations found in the present study. Notwithstanding the foregoing, this study shows a solid correlation that is a starting point to continue investigating and generating more clinical evidence of a correlation between concentrations of drugs in serum and saliva (Mennickent et al. 2007), to have the saliva method as a clinical monitoring alternative, at the same time, that is routinely used in clinical practice to monitor this drug in Mérida-Venezuela, and in Latin American countries. On the other hand, it is necessary to complement pharmacokinetic studies with pharmacogenetic analyzes in various Latin American populations, so that together they allow the implementation of precision medicine (Alvarado et al. 2021b) for personalized dose adjustment, avoiding therapeutic failure, and minimizing the adverse effects of phenytoin and other AEDs (Dwivedi et al. 2016).

Conclusion

It is concluded that there is an association and concordance between the concentrations of phenytoin in serum and saliva. In this sense, the saliva method would be an alternative for the clinical monitoring of phenytoin in children and older adults.

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