ORIGINAL ARTICLE

Estimation of Free Phenytoin Concentration in Critically Ill Patients with Hypoalbuminemia: Direct-measurement vs Traditional Equations

Premila M Wilfred[1], Sumith Mathew[2], Binila Chacko[3], Ratna Prabha[4], Binu Susan Mathew[5]

Abstract

Background: In critically ill patients with low albumin, dose individualization of phenytoin is a challenge. The currently used Sheiner–Tozer equation does not accurately predict the free phenytoin concentration in serum and can result in incorrect dose modifications. The best measure to advocate in these patients is the direct-measurement of free phenytoin concentration.

Aims and objectives: Phenytoin exhibits complex pharmacokinetics, requiring careful therapeutic drug monitoring. This study aimed to compare the accuracy of the established Sheiner–Tozer calculation method against the direct-measurement of free phenytoin concentration in serum by high performance liquid chromatography in critically ill patients with low albumin.

Materials and methods: Blood specimens for direct-measurement of both total and free phenytoin concentration were obtained from 57 patients with hypoalbuminemia monitored in the intensive care unit.

Results: The median [inter-quartile range (IQR)] for Sheiner–Tozer equation calculated total phenytoin concentration and direct-measured total was 17.14 (10.63–24.53) and 9.82 (6.02–13.85) μg mL^{-1}, respectively. Approximately 53 and 5% of patients were found to be subtherapeutic and supratherapeutic for direct-measured total phenytoin concentrations, respectively. In contrast, on applying the Sheiner–Tozer calculation, 23 and 40% had subtherapeutic and supratherapeutic concentrations, respectively, for total phenytoin concentration. The median (IQR) for direct-measured, routine and Sheiner–Tozer equation calculated free phenytoin concentration were 1.92 (1.06–2.76), 0.98 (0.60–1.39), and 1.71 (1.06–2.45) μg mL^{-1}, respectively. Only 45.7% of patients were in agreement with respect to the therapeutic category when direct-measured free was compared against routine calculation free.

Conclusion: In patients with low albumin, free phenytoin concentration based on the Sheiner–Tozer corrected equation accurately classified patients based on their therapeutic category of free phenytoin in 73.7% of patients. Hence, for individualization of phenytoin dosage in critically ill patients with low albumin, we recommend direct-measurement of free phenytoin concentration.

Keywords: Critical care, Free phenytoin, Hypoalbuminemia, Sheiner–Tozer equation.

Indian Journal of Critical Care Medicine (2022): 10.5005/jp-journals-10071-24235

Introduction

Epilepsy is a common clinical condition which has a considerable impact on an individual's quality of life. According to the 2019 World Health Organization's statement, 50 million people are affected by epilepsy globally. Furthermore, approximately 8–10% of the world population can experience a seizure in their lifetime; making this non-communicable disease, a common neurological condition worldwide.[1]

Phenytoin, an effective antiseizure drugs has been used for more than 80 years to achieve and maintain seizure control.[2,3] However, in clinical care, the complex pharmacokinetic properties of phenytoin make dosing of phenytoin an extreme challenge to the treating clinician. Phenytoin exhibits non-linearity in its elimination pharmacokinetics and is a broad-spectrum inducer of cytochrome P450,[4,5] thereby interacting with co-administered drugs.[6,7] Second, 90% of phenytoin is bound to albumin,[8] the remaining 10% unbound or free proportion of phenytoin is identified as the decisive component with regard to the clinical efficacy and toxic effects of phenytoin.[9] Third, total phenytoin concentration has a narrow therapeutic range of 10–20 μg mL^{-1} with a comparable limited free phenytoin concentration in the range 1–2 μg mL^{-1} (total phenytoin concentration/10).[10] Despite these serious concerns with phenytoin, it has the best documented relationship between plasma concentration and clinical efficacy, compared to many anticonvulsant drugs.

Treatment decisions with phenytoin are more complex in critical care patients, who are prone to develop hypoalbuminemia.[11–13] A fall in serum albumin can alter phenytoin binding, potentially raising free phenytoin concentration to toxic levels. Other factors such as old age, altered hepatic functions, uremia,

[1,2,4,5]Department of Pharmacology and Clinical Pharmacology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India

[3]Medical Intensive Care Unit, Christian Medical College and Hospital, Vellore, Tamil Nadu, India

Corresponding Author: Binu Susan Mathew, Department of Pharmacology and Clinical Pharmacology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India, Phone: +91 04162282721, e-mail: binusmathew@cmcvellore.ac.in

How to cite this article: Wilfred PM, Mathew S, Chacko B, Prabha R, Mathew BS. Estimation of Free Phenytoin Concentration in Critically Ill Patients with Hypoalbuminemia: Direct-measurement vs Traditional Equations. Indian J Crit Care Med 2022;26(6):682–687.

Source of support: Nil

Conflict of interest: None

hypercholesterolemia, and sepsis frequently coexist in the critically ill, which will further be altering the free fraction of the drug and warranting heightened clinical vigilance while monitoring.[14] Protein binding of phenytoin in patients with diabetics is also found to be lower, when compared with patients who are non-diabetic.[15] For this reason, in critical care, therapeutic drug monitoring of total phenytoin is routinely performed by the treating clinician.[16] However, the estimation of total phenytoin concentration which is inclusive of the bound and free phenytoin may provide discrepant results in comparison with the biologically active free phenytoin concentration.[17,18]

A common approach toward this problem is a simple "routine calculation method," dividing the total phenytoin concentration by 10, a method that assumes 10% of total phenytoin to be available in the unbound form. However, in the critically ill patient, free phenytoin concentration could possibly be much higher.[10] Alternatively, the standard mathematical algorithm, "Sheiner–Tozer equation" incorporates the albumin concentration with the direct-measured total phenytoin concentration for calculating the corrected phenytoin concentration.[19,20] Despite being decades old, the accuracy of these equations has been questioned by recent researchers.[21–23] In this situation, the direct-measurement of free phenytoin concentration in serum, especially in the critically ill could be essential.[24]

In this study, we compared direct-measured free phenytoin concentration with calculated free phenytoin concentration, using the two following methods: (i) The routine method in which the free phenytoin is estimated to be 10% of the total phenytoin and (ii) The Sheiner–Tozer calculated method in critically ill patients with low albumin.

Materials and Methods

Study Design

This prospective study was conducted in the Clinical Pharmacology Unit and the Medical Intensive Care Unit – Division of Critical Care – Christian Medical College, Vellore, Tamil Nadu, India from December 2016 to August 2018. The study approval was obtained from the Institutional Review Board (IRB Minute No. 10321, dated 12 October 2016) and was performed in accordance with the ethical principles of the updated version of Declaration of Helsinki.

Patient Recruitment and Analysis

A total of 57 patients, aged 18 years and above, who presented with seizures and were managed with phenytoin (loading and maintenance dose) were recruited into the study; after obtaining an informed consent from a first-degree relative. Pregnant mothers were excluded from the study. A record of the suspected diagnosis; concomitant illness; co-medications; renal and hepatic parameters were maintained. In the patients who had a serum albumin below 3.5 gm dL^{-1} and after 3 days of initiation of phenytoin, a single trough blood specimen (5 mL) was collected. The blood specimens were centrifuged at room temperature for 5 minutes at 13,000 rpm. The supernatant serum was transferred and stored at –20°C temperature until analysis. The serum was separated to be used for total phenytoin (600 µL) and free phenytoin (500 µL) estimation. To separate the specimen for free phenytoin, 500 µL of serum was filtered through an ultrafilter (Amicon Centrifree)[25,26] and centrifuged at 13,000 rpm for 30 minutes, at 4°C. This was stored at –20°C for the estimation of free phenytoin, for analysis within 1 week of collection.

High-performance Liquid Chromatography Measurement for Total Phenytoin Concentration

Phenytoin pure powder (5,5-diphenyl hydantoin sodium salt >99%) was obtained from Sigma–Aldrich Incorporated and nevirapine powder were used as the internal standard for the assay.

The assay was developed and validated using an automated injector high-performance liquid chromatography, LC-2010CHT with UV detector at 214 nm.

The samples for measurement of total phenytoin were extracted by simple protein precipitation using acetonitrile.

The inter-day imprecision (calculated as % coefficient of variation) for total phenytoin concentration of 3.03 and 22.85 µg mL^{-1} was 0 and 4.3%, respectively.

High-performance Liquid Chromatography Measurement for Free Phenytoin Concentration

Free phenytoin concentrations were estimated after filtration of proteins from serum using an ultrafilter. The intraday imprecision for free phenytoin concentrations of 0.28 and 2.36 µg mL^{-1} was 6.1 and 4.5%, respectively. The lower limit of quantification (LLOQ) for the total and free phenytoin was 2.76 and 0.06 µg mL^{-1}, respectively.

In this study, total phenytoin concentration was estimated by the following two methods: (i) "Direct-measured total phenytoin concentration" and (ii) Calculated "Sheiner–Tozer total phenytoin concentration," a corrected value that predicts the effect of albumin on the phenytoin concentration. Free phenytoin concentration was estimated by the following three methods: (i) "Routine calculation method" ("Direct-measured total phenytoin concentration/10"), (ii) The "Sheiner–Tozer free phenytoin concentration," and (iii) "Direct-measured free phenytoin concentration." "Routine calculation" and "Sheiner–Tozer equation" for calculating free phenytoin used the value obtained from the direct total measured phenytoin concentration, in separate equations.

Statistical Analysis

The trough concentrations of total and free serum phenytoin were determined by visually inspecting the data. The R software, version 3·2·1, was used for further data analysis. The patients were then stratified into three categories for phenytoin concentration with respect to one method. The total and free phenytoin concentration was labeled as subtherapeutic for <10 and <1 µg mL^{-1}, respectively; therapeutic for 10–20 and 1–2 µg mL^{-1}; and supratherapeutic for >20 and >2 µg mL^{-1}, respectively. The direct-measured values for both total and free were used as the standard for comparison against each calculation method. The level of agreement between the direct-measured phenytoin concentration and the phenytoin concentration derived from calculated methods were analyzed to the respective therapeutic category. Free phenytoin concentration with regard to albumin, bilirubin, and comedication was also evaluated.

Results

Approximately 58% of patients who were diagnosed to have either meningoencephalitis, intracranial bleed or cortical venous thrombosis participated in this study. The baseline demographic characteristics of the patients are summarized in Table 1.

Table 1: Characteristics of the patients ($n = 57$) at baseline values are *n* (%) or median (IQR)

Characteristic	*Values*
Age (years)	37 (29.0–56.0)
Sex	
Male–Number (%)	36 (63.2)
Female–Number (%)	21 (36.8)
Biochemistry parameters	*Median ± IQR*
Creatinine (mg dL^{-1})	0.83 (0.59–1.48)
Total bilirubin (mg dL^{-1})	0.54 (0.38–0.72)
Direct bilirubin (mg dL^{-1})	0.26 (0.15–0.40)
Total protein (gm dL^{-1})	5.70 (5.30–6.30)
Total albumin (gm dL^{-1})	2.50 (2.20–2.80)
Co-medications	*Number*
Monotherapy with phenytoin (*n*)	30
One additional antiepileptic drug (*n*)	10
Two additional antiepileptic drugs (*n*)	8
Three or more additional antiepileptic drugs (*n*)	9
MICU admission diagnosis	*Number of patients (%)*
Meningoencephalitis	17 (29.8)
Intracranial bleed/infarct/CVT	16 (28.1)
Poisoning	5 (8.77)
Epilepsy	4 (7.02)
Systemic lupus erythematosus	2 (3.51)
Others (malignancy, infections)	13 (22.8%)

Total Phenytoin Concentration

The median and IQR concentration for "direct-measured total phenytoin concentration" and "Sheiner–Tozer calculated total phenytoin concentration" was 9.8 (6.0–13.9) and 17.14 (10.63–24.53) μg mL^{-1}, respectively. The interpatient variability (calculated as % CV) for both, direct-measured total and Sheiner–Tozer total phenytoin concentration ranged 62–64%.

With respect to the direct-measured total phenytoin concentration in 57 patients, 53% (30/57) were subtherapeutic, 42% were therapeutic (24/57), and only 5% were supratherapeutic. In contrast, using the Sheiner–Tozer equation, 23%(13) patients were classified as subtherapeutic, 37%(21) as therapeutic, and 40% (23) patients were included in the supratherapeutic category. The correlation between the "direct-measured total phenytoin concentration" and the "Sheiner–Tozer calculated total phenytoin concentration" using the Pearson correlation coefficient (R^2) was 0.90.

The direct-measured total phenytoin concentration was first stratified into below, within, and above the therapeutic range for the total phenytoin concentration. This was compared against the concomitant Sheiner–Tozer calculated total phenytoin concentrations. The agreement between the two methods was 43, 25, and 100% for the subtherapeutic, therapeutic, and supratherapeutic categories, respectively.

Free Phenytoin Concentration

The median (IQR) by routine calculation, direct-measured and Sheiner–Tozer calculated free phenytoin was 0.98 (0.60–1.39), 1.92 (1.06–2.76) and 1.71 (1.06–2.45) μg mL^{-1} respectively. The interpatient variability (CV%) of the direct-measured free phenytoin concentration was 76.5%.

Direct-measured Free vs Routine Calculation Free

Of the 57 patients, 23[13], 33.3[19], and 44%[25] patients were subtherapeutic, therapeutic, and supratherapeutic, respectively, with respect to the direct-measured free phenytoin concentration (Fig. 1). The direct-measured free phenytoin and direct-measured total phenytoin concentration is represented in Figure 2. Approximately 53, 42, and 5% were subtherapeutic, therapeutic, and supratherapeutic, respectively, with respect to the routine calculation free phenytoin concentration.

All 13 patients who were subtherapeutic by direct-measured free phenytoin concentrations were subtherapeutic by the routine calculation free as well. Of the 19 in the therapeutic range using direct-measured free, only 52.6%[10] remained therapeutic whereas

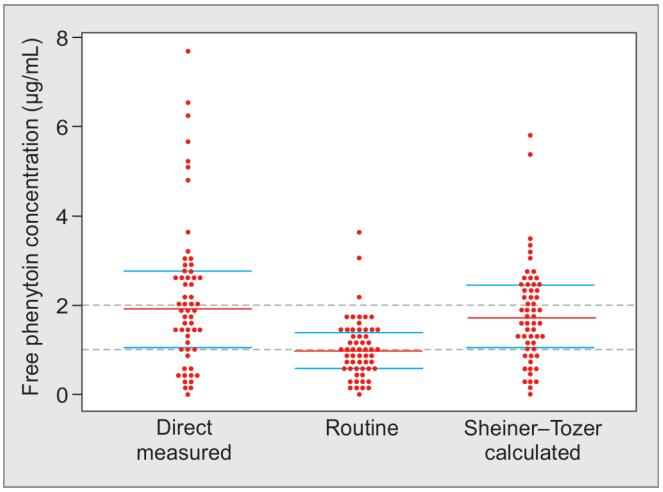

Fig. 1: Beeswarm plot comparing the distribution of free phenytoin concentration by the three methods with respect to the therapeutic range of free phenytoin concentration. The dashed lines represent the upper and lower limit of the therapeutic range of free phenytoin concentration. The bold dark and thin gray lines depict the median and the interquartile ranges of free phenytoin concentration by the three methods, respectively

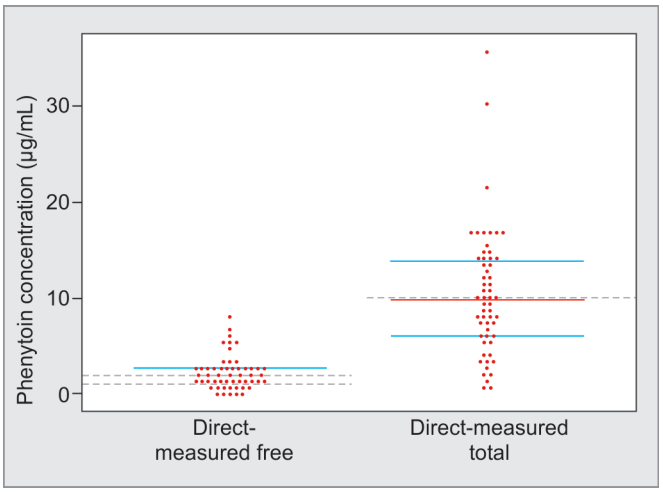

Fig. 2: Beeswarm plot comparing the distribution of measured total and free phenytoin concentrations by HPLC. The dashed lines represent the upper and lower limit of the therapeutic range of free phenytoin concentration. The bold dark and thin gray lines depict the median and the interquartile ranges of free phenytoin concentration by the three methods, respectively

47.4%[9] would be falsely reported as subtherapeutic by the routine calculation free. Among the 25 patients, in the supratherapeutic category by direct-measured free, only 12%[3] were supratherapeutic using the routine calculation free, whereas 56[14] and 32%[8] were in the therapeutic and subtherapeutic categories, respectively. Overall, 26/57 patients (45.7%), were in agreement with respect to the therapeutic category when direct-measured free was compared against routine calculation free.

The Pearson correlation coefficient (R^2) between direct-measured and routine calculation free phenytoin concentration was 0.64.

Direct-measured Free vs Sheiner–Tozer Free Phenytoin Concentration

Approximately 23, 37, and 40% were subtherapeutic, therapeutic, and supratherapeutic with respect to the Sheiner–Tozer free phenytoin concentration. Among the 13 patients who were subtherapeutic with direct-measured free, 12 were in the same category with the Sheiner–Tozer free. Among the 19 patients in the therapeutic range with direct-measured free, 68%[13] were therapeutic with Sheiner–Tozer free, but 32%(6) were supratherapeutic using the Sheiner–Tozer free calculation. Among the 25 patients who were supratherapeutic with direct-measured free, 68%[17] were supratherapeutic using the Sheiner–Tozer free equation. Overall, 42/57 patients (73.7%), were in agreement with respect to the therapeutic category when direct-measured free was compared against Sheiner–Tozer free (Fig. 3).

Correlation (R^2) for direct-measured free and Sheiner–Tozer calculated free concentrations was 0.637. The Bland–Altman plot is represented in Figure 4.

Free Phenytoin Concentration in Relation to Albumin

The plasma albumin of patients enrolled in the study ranged from 1.3 to 3.3 gm dL^{-1} with a median (IQR) of 2.50 (2.20–2.80). Seven out of 57 patients had a very low albumin (<2 gm%). While comparing direct-measured free (gold standard) and Sheiner–Tozer free concentration, only one among the seven patients was not in the similar therapeutic category. However, there was no agreement

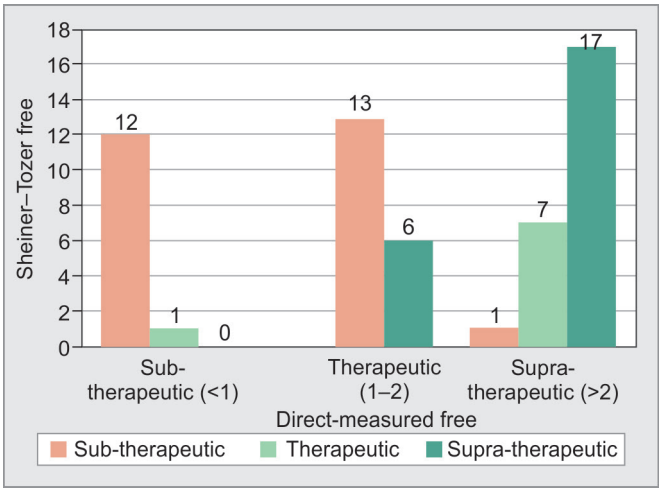

Fig. 3: Bar graphs representing variation of Sheiner–Tozer free with respect to direct-measured free phenytoin concentrations. The data was derived by grouping of patients into subtherapeutic, therapeutic and supratherapeutic range of free phenytoin concentration

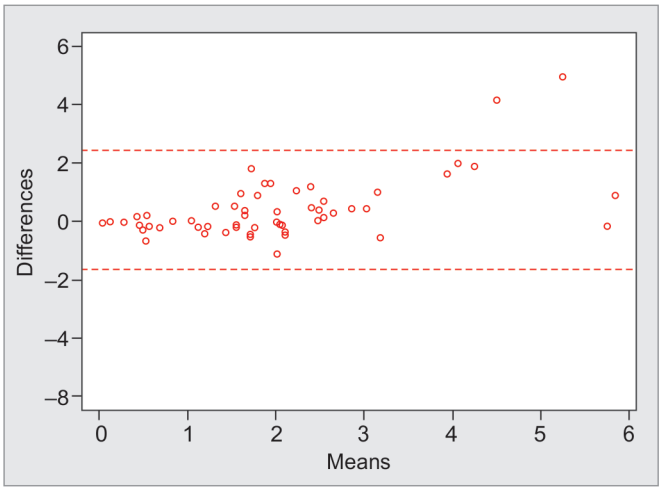

Fig. 4: Bland–Altman plot to determine the agreement between direct-measured free phenytoin and Sheiner–Tozer calculated free phenytoin. The thin line represents the bias (mean difference) and the bold lines represent ±2 SD of bias

between direct-measured free and the routine free concentration in any patient. In patients with hypoalbuminemia, the correlation between direct-measured free phenytoin concentration and serum albumin was 0.007 (R^2).

When albumin was categorized into severe (<2 gm dL^{-1}) and moderate hypoalbuminemia (>2 gm dL^{-1}), the median (IQR) for free phenytoin concentration was 2.89 (1.85–3.24) and 1.8 (0.95–2.63) µg mL^{-1} for the severe- and moderate-hypoalbuminemic groups, respectively.

Free Phenytoin Concentration with respect to Co-medications and Bilirubin

Among the 57 patients recruited, 11 received the antiepileptic drug valproic acid (known for displacing phenytoin and altering drug metabolism). The median (IQR) of direct-measured free phenytoin concentration of patients with and without valproate was 2.8 (2.4–5.2) and 1.7 (1.03–2.6) µg mL^{-1}, respectively.

Only 11 patients had a high total bilirubin >1.2 mg dL^{-1}. There was a poor correlation (R^2 = 0.37) between the free fraction of phenytoin and bilirubin.

Discussion

The challenge faced by the clinician when treating patients with phenytoin is primarily related to "vital dosing decisions," to be made in the critical care setting.[27,28] Application of the Sheiner–Tozer equation, a model presumably driven by a single-factor albumin, may provide inaccurate calculated free phenytoin concentrations.[29–31]

To our knowledge, in India, there have been very few studies, evaluating the clinical utility of the Sheiner–Tozer equation, comparing the former with the direct-measurement of total phenytoin in critically ill patients with low albumin. Buckley et al. have reported Sheiner–Tozer equation overestimating free phenytoin concentration compared to the direct-measured free concentration.[32]

Hong et al. reported the mean difference between the direct-measured and Sheiner–Tozer calculated free phenytoin

concentrations as 0.65 ± 0.88 μg mL^{-1}; (95% CI 1.11–2.41) which was similar to the mean [standard deviation (SD)] difference between direct-measured and Sheiner–Tozer calculated free phenytoin concentration (0.40 ± 1.03 μg mL^{-1}) observed in our study. The mean (SD) serum albumin concentration was comparable to the serum albumin concentration in our patients (3.3 ± 0.8 vs 2.45 ± 0.44).[30]

In a retrospective study, conducted by Krasowski and colleagues, the correlation between the direct-measured free phenytoin and Sheiner–Tozer free ($r = 0.79$) was similar to our study ($r = 0.798$). In addition, the concordance between Sheiner–Tozer calculated total and direct free phenytoin concentration in the therapeutic group was 68% which was also similar to our study.[33]

In many institutions, in developing countries, the method of choice while treating with phenytoin depends on the clinical condition of the patient and specifically, the acuteness of the illness. However, many hospitals such as our hospital, in India and globally, continue to depend on the Sheiner–Tozer equation for individualizing phenytoin dosage in patients with low albumin admitted in critical care. As confirmed in our study, an overall of 73.7% patients would be rightly categorized according to the therapeutic concentration category when managed based on the Sheiner–Tozer calculation, rather than the direct free measurement of phenytoin. Specifically, free phenytoin concentration if calculated using the Sheiner–Tozer equation is more acceptable for the subtherapeutic range. Whereas for those in the therapeutic or supratherapeutic category, approximately 68% of patients were in the correct therapeutic category, when compared against the direct-measurement of free phenytoin.

Although these equations can offer some guidance, identification of coexistent factors and direct-measurement of free phenytoin concentration would substantially improve quality of care in the critically ill.[34] Furthermore, this would improve patient-centric therapy and aid in deploying additional antiseizure combination therapy in the non-responsive epilepsy patient.

Direct-measurement of free phenytoin concentration measurement is not without difficulty. The procedure is labor-intensive, expensive, time consuming, and tedious, requiring an additional ultracentrifugation step, making this facility available only in specialized centers.[33,35]

Conclusion

In conclusion, our study demonstrates strong evidence supporting the direct-measurement of free phenytoin concentration for individualizing phenytoin dosage in patients with low albumin in a critical care setting. However, in hospitals where there is a limitation of this facility, the conventional Sheiner–Tozer calculation equation may be applied while considering coexistent conditions that could potentially affect free phenytoin concentration. For broader clinical application, clinical insight from the treating clinician is imperative.

Orcid

Premila M Wilfred https://orcid.org/0000-0003-0211-2834
Sumith Mathew https://orcid.org/0000-0002-2993-2392
Binila Chacko https://orcid.org/0000-0002-1609-2208
Ratna Prabha https://orcid.org/0000-0002-0318-9617
Binu Susan Mathew https://orcid.org/0000-0001-6532-1559

References

1. World Health Orgainzation. Global epilepsy report 2019.WHO. Available from: https://www.who.int/mental_health/neurology/epilepsy/report_2019/en/
2. Zafar SN, Khan AA, Ghauri AA, Shamim MS. Phenytoin versus leviteracetam for seizure prophylaxis after brain injury–a meta-analysis. BMC Neurol 2012;12(1):30. DOI: 10.1186/1471-2377-12-30.
3. Masapu D, Gopala Krishna KN, Sanjib S, Chakrabarti D, Mundlamuri RC, Manohar N, et al. A comparative study of midazolam and target-controlled propofol infusion in the treatment of refractory status epilepticus. Indian J Crit Care Med 2018;22(6):441–448. DOI: 10.4103/ijccm.IJCCM_327_17.
4. Giancarlo GM, Venkatakrishnan K, Granda BW, von Moltke LL, Greenblatt DJ. Relative contributions of CYP2C9 and 2C19 to phenytoin 4-hydroxylation *in vitro*: inhibition by sulfaphenazole, omeprazole, and ticlopidine. Eur J Clin Pharmacol 2001;57(1):31–36. DOI: 10.1007/s002280100268.
5. Thakkar AN, Bendkhale SR, Taur SR, Gogtay NJ, Thatte UM. Association of CYP2C9 polymorphisms with phenytoin toxicity in Indian patients. Neurol India 2012;60(6):577–580. DOI: 10.4103/0028-3886.105189.
6. Anderson GD. Pharmacogenetics and enzyme induction/inhibition properties of antiepileptic drugs. Neurology 2004;63(10 Suppl. 4):S3–S8. DOI: 10.1212/wnl.63.10_suppl_4.s3.
7. Iwamoto T, Kagawa Y, Naito Y, Kuzuhara S, Okuda M. Clinical evaluation of plasma free phenytoin measurement and factors influencing its protein binding. Biopharm Drug Dispos 2006;27(2):77–84. DOI: 10.1002/bdd.486.
8. Jain S, Gautam V, Naseem S. Acute-phase proteins: as diagnostic tool. J Pharm Bioallied Sci 2011;3(1):118–127. DOI: 10.4103/0975-7406.76489.
9. Levine M, Chang T. Therapeutic drug monitoring of phenytoin. Rationale and current status. Clin Pharmacokinet 1990;19(5):341–358. DOI: 10.2165/00003088-199019050-00001.
10. Patsalos PN, Berry DJ, Bourgeois BFD, Cloyd JC, Glauser TA, Johannessen SI, et al. Antiepileptic drugs–best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia 2008;49(7):1239–1276. DOI: 10.1111/j.1528-1167.2008.01561.x.
11. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. Br J Anaesth 2000;85(4):599–610. DOI: 10.1093/bja/85.4.599.
12. von Winckelmann SL, Spriet I, Willems L. Therapeutic drug monitoring of phenytoin in critically ill patients. Pharmacotherapy 2008;28(11):1391–400. DOI: 10.1592/phco.28.11.1391.
13. Montgomery MC, Chou JW, McPharlin TO, Baird GS, Anderson GD. Predicting unbound phenytoin concentrations: effects of albumin concentration and kidney dysfunction. Pharmacother J Hum Pharmacol Drug Ther 2019;39(7):756–766. DOI: 10.1002/phar.2273.
14. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med 2017;43(3):304–377. DOI: 10.1007/s00134-017-4683-6.
15. Doucet J, Fresel J, Hue G, Moore N. Protein binding of digitoxin, valproate and phenytoin in sera from diabetics. Eur J Clin Pharmacol 1993;45(6):577–579. DOI: 10.1007/BF00315318.
16. Pillai LV, Vaidya N, Khade AD, Hussainy S. Variability of serum phenytoin levels in critically ill head injured patients in intensive care unit. Indian J Crit Care Med 2008;12(1):24–27. DOI: 10.4103/0972-5229.40946.
17. Dasgupta A. Usefulness of monitoring free (unbound) concentrations of therapeutic drugs in patient management. Clin Chim Acta 2007;377(1–2):1–13. DOI: 10.1016/j.cca.2006.08.026.
18. Peterson GM, McLean S, Aldous S, Witt RV, Millingen KS. Plasma protein binding of phenytoin in 100 epileptic patients. Br J Clin Pharmacol 1982;14(2):298–300. DOI: 10.1111/j.1365-2125.1982.tb01981.x.
19. Interpatient and intrapatient variability in phenytoin protein binding. Abstract. Europe PMC. Available from: https://europepmc.org/article/med/12021629.

20. Tobler A, Hösli R, Mühlebach S, Huber A. Free phenytoin assessment in patients: measured versus calculated blood serum levels. Int J Clin Pharm 2016;38(2):303–309. DOI: 10.1007/s11096-015-0241-x.
21. Chesher D. Calculated free phenytoin and albumin adjusted total phenytoin versus measured free phenytoin. Pathology 2019;51:S108. https://doi.org/10.1016/j.pathol.2018.12.303.
22. Kiang TKL, Ensom MHH. A comprehensive review on the predictive performance of the Sheiner–Tozer and derivative equations for the correction of phenytoin concentrations. Ann Pharmacother 2016;50(4):311–325. DOI: 10.1177/1060028016628166.
23. Barra ME, Phillips KM, Chung DY, Rosenthal ES. A novel correction equation avoids high-magnitude errors in interpreting therapeutic drug monitoring of phenytoin among critically ill patients. Ther Drug Monit 2020;42(4):617–625. DOI: 10.1097/FTD.0000000000000739.x.
24. Svensson CK, Woodruff MN, Baxter JG, Lalka D. Free drug concentration monitoring in clinical practice. Rationale and current status. Clin Pharmacokinet 1986;11(6):450–469. DOI: 10.2165/00003088-198611060-00003.
25. Tacker D, Robinson R, Perrotta PL. Abbott ARCHITECT iPhenytoin assay versus similar assays for measuring free phenytoin concentrations. Lab Med 2014;45(2):176–181. DOI: 10.1309/lm28b9dsrjcbcwwj.
26. Kilpatrick CJ, Wanwimolruk S, Wing LM. Plasma concentrations of unbound phenytoin in the management of epilepsy. Br J Clin Pharmacol 1984;17(5):539–546. DOI: 10.1111/j.1365-2125.1984.tb02387.x.
27. Imam SH, Landry K, Kaul V, Gambhir H, John D, Kloss B. Free phenytoin toxicity. Am J Emerg Med 2014;32(10):1301.e3–4. DOI: 10.1016/j.ajem.2014.03.036.
28. Muñoz–Pichuante D, Villa Zapata L, Cabrera S, Lagos X, Grandjean J. Dosage of phenytoin in neurocritical patients using Bayesian algorithms: a pilot study. Drug Metab Pers Ther 2019;18:34(4):/j/dmdi.ahead-of-print/dmpt-2019-0015/dmpt-2019-0015.xml. DOI: 10.1515/dmpt-2019-0015.
29. Javadi S-S, Mahjub R, Taher A, Mohammadi Y, Mehrpooya M. Correlation between measured and calculated free phenytoin serum concentration in neurointensive care patients with hypoalbuminemia. Clin Pharmacol Adv Appl 2018;10:183–190. DOI: 10.2147/CPAA.S186322. eCollection 2018.
30. Hong J-M, Choi Y-C, Kim W-J. Differences between the measured and calculated free serum phenytoin concentrations in epileptic patients. Yonsei Med J 2009;50(4):517–520. DOI: 10.3349/ymj.2009.50.4.517.
31. Parikh L, MacLaren R. The predictive performances of equations used to estimate unbound phenytoin concentrations in a medical ICU population and the impact of exogenous albumin administration. J Crit Care 2018;44:95–100. DOI: 10.1016/j.jcrc.2017.10.028.
32. Buckley MS, Reeves BA, Barletta JF, Bikin DS. Correlation of free and total phenytoin serum concentrations in critically pll patients. Ann Pharmacother 2016;50(4):276–281. DOI: 10.1177/1060028015627468.
33. Krasowski MD, Penrod LE. Clinical decision support of therapeutic drug monitoring of phenytoin: measured versus adjusted phenytoin plasma concentrations. BMC Med Inform Decis Mak 2012;12:7. DOI: 10.1186/1472-6947-12-7.
34. Soriano VV, Tesoro EP, Kane SP. Characterization of free phenytoin concentrations in end-stage renal disease using the Winter–Tozer equation. Ann Pharmacother 2017;51(8):669–764. DOI: 10.1177/1060028017707541.
35. McMillin GA, Juenke J, Dasgupta A. Effect of ultrafiltrate volume on determination of free phenytoin concentration. Ther Drug Monit 2005;27(5):630–633. DOI: 10.1097/01.ftd.0000173373.12569.c7.