# CORRELATION OF FREE FRACTION OF PHENYTOIN AND PLASMA ALBUMIN LEVEL IN HEAD TRAUMA PATIENTS

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## ABSTRACT

Preliminary data suggests that vital physiologic support measures and free fraction of phenytoin are altered following head trauma. Therefore, we conducted a prospective, randomized controlled study to determine the correlation of albumin concentration and unbound phenytoin plasma concentration following head injury. Ten adult head trauma patients in the neurosurgical intensive care unit receiving phenytoin for the seizure prophylactic treatment were studied for their free and total plasma phenytoin concentration in peak and trough times and their respective albumin concentration. Free and total phenytoin levels were determined by both liquid chromatography and flurescence polarization immunoassay (Eclair) of plasma samples after ultrafiltration and deproteinization. No significant difference was found in the plasma concentration measured with HPLC or FPIA while a marked correlation was noted between plasma albumin and free phenytoin concentration ($r^2$= 0.85). The total and free phenytoin concentrations were not significantly correlated ($r^2$=0.60). A remarkable difference ($P<0.05$) was noticed when doses in patients were adjusted on the basis of total plasma phenytoin and calculated plasma phenytoin adjusted for serum albumin. Therefore, therapeutic monitoring in neurosurgical patients receiving phenytoin should be performed on the basis of pharmacologically active component (free fraction), rather than total phenytoin which is presently performed in the clinics.

**Keywords:** Free fraction, Phenytoin, Albumin, Head trauma, Correlation

## INTRODUCTION

Phenytoin is commonly administered as an anticonvulsant to critically ill patients for seizure prophylaxis and treatment. It exhibits non-linear pharmacokinetic charactristic and requires frequent plasma monitoring and dose adjustments (1,2). Based on a previous study, it is often difficult to achieve therapeutic levels in patients with neurologic trauma using the recommended phenytoin dosing strategies (3). A variaty of mechanisms have been proposed to explain this phenomenon, including stress-related increase in hepatic metabolism (4), increase in clearance due to hypermetabolic or hypercatabolic state during head trauma, and an increase in clearance of unbound phenytoin resulting in increased phenytoin total clearance (1,2). Under normal conditions, about 90% of phenytoin is bound to plasma proteins, primarily albumin which constitutes 85% of binding substrate (5). The binding of phenytoin to albumin is also reported to be altered in the critically ill patients following trauma which might change the free phenytoin fraction (6). There are also reports on preference of monitoring of both total and free phenytoin concentrations in head trauma patients (1). Measurement of unbound free fraction of phenytoin instead of total drug has also been recommended for ICU patients (7,8). This investigation was designed for further evaluation of this phenomenon and to study possible existance of the correlation between free phenytoin and albumin concentration in our subjects.

## MATERIAL AND METHODS

*Selection of Subjects*

Ten adult head trauma patients who were admitted to neurosurgical intensive care unit and

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received phenytoin for the seizure prophylactic treatment were enrolled in this study. The inclusion criteria for these patients were, severe head injury, over 18 years of age and Glasgow Coma Scale (GCS) score of less than 8. Patients with bradycardia, second or third degree heart block, clinically significant hypotension, hepatic or renal disease (total bilirubin>3mg/dL and/or ALT>120µg/l and/or plasma creatinine>2 mg/dL were excluded. Subjects who had previous history of receiving phenytoin or any other medications known to interfere with phenytoin protein binding or metabolism and patients who required concomitant use of valporic acid, phenobarbital, sulfonamides or theophylline were also excluded from the study. The study protocol was approved by the Institutional Review Board at Tehran University of Medical Sciences.

*Drug administration and blood sampling*

Phenytoin sodium (Daru-Pakhsh,50mg/ml) diluted in normal saline to a concentration of 25mg/ml and all intravenous doses were administered at rate of no faster than 25mg/min. Maintenance dose of phenytoin sodium was administered for the first day of head trauma and vital signs were monitored hourly while the patients remained at the neurosurgical intensive care unit. Blood samples were obtained from forearm vein of the patients at 30 minutes after the end of infusion to determine peak concentrations and 30 minutes before the next dose to determine trough concentrations. Venous blood samples (5ml) were obtained and collected in heparinized tube and centrifuged at room temprature for 15 minutes at 3000 rpm/min to obtain plasma. An aliqut of plasma was filtered through suitable ultrafilter (Amicon centrifree, cut off=5000 Dalton) for preparation of free fraction.

*Drug Assay Procedure*

Extraction of plasma samples for total phenytoin was performed by deproteinization of samples with the same volume of acetonitrile. 50 µL of supernatant was injected into the HPLC column for determination of total plasma phenytoin, and 50 µL of ultrafiltered sample was directly injected into HPLC column to determine free phenytoin. Analysis was performed by using a 600E high perssure pump, and a 486 UV spectrophotometer, a 746 data module (all from waters). The samples were itroduced to a Techsphere C8 column (3 µm,150×4.6 mm) through a rheodyne 7725 injector fitted by a 50µL loop. Acetonitrile:water (73.4:26.6, pH=2.5) was used as mobile phase with a flow rate of 1.4 ml/min and the eluent was monitored at 210 nm. Column temprature was maintained at 30ºC during the assay. Total plasma phenytoin also were determined by Merck VITALAB Eclair that works based on flurescence polarization immunoassay.

*Pharmacokinetic calculations*

Equation (I), was used to calculate unbound fraction of phenytoin on the first day and day after.

Normal albumin concentration was assumed to be 4.3g/dl. The actual volume of distribution ($V_d$) was estimated from the serum albumin concentration according to Equation (II) (9). Winter-Tozer method (Equation III) was used to calculate adjusted phenytoin concentration (10).

$F_{up}= 1/(1+2.1\times Alb)$ (Equation I)

$Vd_{(L/kg)}=2.8/Alb$ (Equation II)

$C_{normal}=C_{observed}/(0.2\times Alb+0.1)$ (Equation III)

## RESULTS AND DISCUSSION

Free and total phenytoin levels were determined by both liquid chromatography and flurescence polarization immunoassay (Eclair). No significant difference was noted in the plasma concentration measured with HPLC or Eclair. Patients demographic data are provided in table 1. Albumin variation , unbound fraction on the day of one and later, total and free phenytoin concentration for patients enrolled at the study are provided in tables 2 and 3. In seven of ten patients, an overall 0.5g/dL decline in albumin concentration was noted. This was in agreement with previous reports (11). One of the patients demonstrated an increase in albumin concentration and a decrease in unbound fraction, and two patients did not show any variation in plasma albumin concentration. Although almost 50% of patients received equal doses of phenytoin, there were variation in their total phenytoin plasma concentration which could be due to interindividual variations in capacity limited nature of phenytoin metabolism and plasma protein binding.

Based on our findings whenever the total plasma concentrations of phenytoin were subtherapeutic, free phenytoin concentrations were also low. There was a strong inverse correlation between plasma albumin and free phenytoin concentration during the treatment ($r^2$=0.85) (figure I), but total and free phenytoin concentrations were weakly correlated ($r^2$=0.60). Under normal conditions, about 90% of phenytoin is bound to plasma

**Table 1** Patients demographic

| Patient no. | Age (year) | Gender (Male/Female | Weight (kg) | GCS[1] | Diagnosis | Outcome |
|---|---|---|---|---|---|---|
| 1 | 40 | M | 62 | 4 | Frontal Contusion | [3]D/C |
| 2 | 55 | M | 63 | 5 | Trauma- lateral ventricle hemorrhage | [3]D/C |
| 3 | 55 | M | 60 | 5 | Trauma- Epidural bleeding | [3]D/C |
| 4 | 60 | M | 60 | 5 | MVA[2]-ventricle hemorrhage | [3]D/C |
| 5 | 52 | F | 70 | 4 | MVA[2]-subdural hemorrhage | [3]D/C |
| 6 | 41 | M | 65 | 7 | Trauma-epidural hemorrhage | [3]D/C |
| 7 | 24 | M | 65 | 8 | Trauma- interaparanchymal hemorrhage | [3]D/C |
| 8 | 45 | F | 70 | 5 | Truma- subarachnoid hemorrhage | [3]D/C |
| 9 | 50 | F | 70 | 6 | Closed-head injury | [3]D/C |
| 10 | 55 | F | 65 | 5 | Skull fracture | [3]D/C |

[1]Glasgow Coma Scale. [2]Motor vehicle accident. [3]Discharged

**Table 2.** Variation of plasma albumin concentration and unbound fraction of phenytoin

| Patient no. | Albumin Con (g/dL)[a] | Albumin Con (g/dL)[b] | Δ Alb Con (g/dL) | %Free[c] on Day One | %Free[c] on Day after | %Δ Unbound Calculated | %Free by HPLC[d] |
|---|---|---|---|---|---|---|---|
| 1 | 4.00 | 3.40 | -0.60 | 0.106 | 0.123 | 16.0 | 14.6 |
| 2 | 4.30 | 4.30 | 0.00 | 0.099 | 0.099 | 0.0 | 7.2 |
| 3 | 4.00 | 3.70 | -0.30 | 0.106 | 0.114 | 7.5 | 10.1 |
| 4 | 4.10 | 3.70 | -0.40 | 0.104 | 0.114 | 9.0 | 10.4 |
| 5 | 4.10 | 4.20 | 0.10 | 0.104 | 0.102 | -2.0 | 8.2 |
| 6 | 4.00 | 3.60 | -0.40 | 0.106 | 0.117 | 10.0 | 10.6 |
| 7 | 4.10 | 3.60 | -0.50 | 0.104 | 0.117 | 12.5 | 10.6 |
| 8 | 3.40 | 2.70 | -0.70 | 0.123 | 0.149 | 21.0 | 17.1 |
| 9 | 3.70 | 3.20 | -0.50 | 0.114 | 0.129 | 13.1 | 12.3 |
| 10 | 4.30 | 4.30 | 0.00 | 0.099 | 0.099 | 0.0 | 11.1 |
| *Mean* | 4.00 | 3.67 | -0.33 | 0.106 | 0.116 | 8.7 | 11.2 |
| *SD* | 0.27 | 0.51 | 0.28 | 0.007 | 0.015 | 7.5 | 2.8 |

[a]Patients albumin concentration on day one. [b]Patients albumin concentration on day after. [c]Calculated unbound fraction of phenytoin on day one and day after. [d]Percent of unbound fraction of phenytoin measured by HPLC.

**Table 3.** Comparison of Total and free phenytoin concentration and predicted and adjusted Vd

| Patient no. | Administered Dose (mg/d) | Total PHT[a] Conc (mg/L) | Free PHT[a] Conc (mg/L) | Trough Conc (mg/L) | Adjusted Conc (mg/L)[b] | Predicted Vd (L/kg) | Adjusted Vd (L/kg)[c] | Plasma pH |
|---|---|---|---|---|---|---|---|---|
| 1 | 250 | 4.10 | 0.60 | 2.00 | 5.52 | 0.63 | 0.82 | 7.30 |
| 2 | 300 | 5.53 | 0.40 | 1.90 | 5.70 | 0.60 | 0.65 | 7.45 |
| 3 | 300 | 6.92 | 0.70 | 2.95 | 8.20 | 0.65 | 0.75 | 7.30 |
| 4 | 300 | 8.59 | 0.90 | 3.12 | 10.20 | 0.63 | 0.75 | 7.35 |
| 5 | 250 | 4.85 | 0.40 | 2.10 | 5.15 | 0.67 | 0.66 | 7.35 |
| 6 | 250 | 5.20 | 0.55 | 2.21 | 6.30 | 0.65 | 0.77 | 7.34 |
| 7 | 250 | 5.18 | 0.55 | 1.73 | 6.30 | 0.64 | 0.77 | 7.30 |
| 8 | 250 | 5.84 | 1.00 | 2.50 | 9.12 | 0.66 | 1.00 | 7.10 |
| 9 | 300 | 8.90 | 1.10 | 2.40 | 12.00 | 0.63 | 0.87 | 7.30 |
| 10 | 300 | 8.50 | 0.95 | 2.30 | 8.85 | 0.65 | 0.65 | 7.35 |
| *Mean* | 275.00 | 6.36 | 0.72 | 2.32 | 7.73 | 0.64 | 0.77 | 7.31 |
| *SD* | 26.35 | 1.74 | 0.26 | 0.44 | 2.30 | 0.02 | 0.11 | 0.09 |

[a]Total and free phenytoin concentration measured by HPLC. [b]Adjusted total phenytoin concentration based on albumin. [c]Adjusted volume of distribution based on albumin.

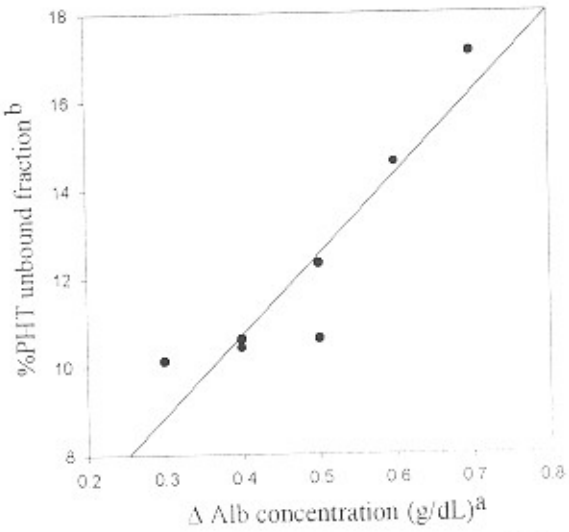


**Figure 1.** Correlation of albumin changes and free fraction of phenytoin. [a]Δ Alb conc. (g/dl)= Patient's albumin concentration- normal albumin concentration. [b]%Phenytoin unbound concentration measured by HPLC.

proteins (primarily to albumin which constitutes 85% of binding substrate). Therefore reduction in albumin concentration observed in head trauma patients in this study have caused variations in free phenytoin plasma concentration.

Since pharmacologically active component is only the free fraction of the drug, this might cause changes in patient's condition. As mentioned above, the correlation between total and free phenytoin was weak. These two observations (a: decrease of albumin concentration during treatment, b: lack of correlation between total and free phenytoin) necessitates measurement of phenytoin free fraction during treatment due to a significant difference between calculated plasma phenytoin level, based on albumin concentration, and the plasma phenytoin concentrations measured by HPLC (Table 1). There are reports on preference of monitoring of both total or free phenytoin concentrations in head trauma patients (1).

Some researchers recommended measurrement of unbound fraction of phenytoin instead of total drug (7,8). This is in concordance with our results on preference of phenytoin free fraction monitoring.

Bouer and co-workers (2) found that although patients with head trauma had statistically sub-therapeutic total phenytoin concentration, free concentration remains in therapeutic level which is in contrast with our results in which our subjects had sub-therapeutic free levels with sub-therapeutic total phenytoin concentration. They attributed higher percentage of unbound phenytoin to hypo-albuminemia in the head trauma patients, whereas patients in this study had variable, insignificant increase in the free fraction of phenytoin. It seems that more than one mechanism are involved in this phenomenon. In addition to increased unbound plasma concentration followed by a decrease in plasma proteins, increased total clearance due to drug interaction (12,9) and increased $V_{max}$ due to the stress-related increase in hepatic metabolism might be involved in this complex phenomenon (4). Two additional studies of phenytoin pharmacokinetics in adult trauma patients have been reported. Boucher and colleagues (13) noted an increased free fraction of phenytoin between day 1 and 7 (median increase 29%) in nine of ten patients during the follow-up period, three of four patients had substantially lower total plasma concentrations than those predicted values. They speculated that an increased metabolism combined with a decreased protein binding was responsible for the lower total phenytoin concentration.

In a subsequent study by the same group (14), an increase of the free fraction and a decrease in total phenytoin concentration were found. In the present study it was noted that critically ill patients often had lower plasma total phenytoin concentration and seven patients demonstrated a decrease in plasma albumin concentration during study followed by an increase in the percent of free fraction. These findings are consistent with the results of previous studies indicating that higher doses of phenytoin than those recommended are necessary to achieve therapeutic plasma concentration following neurologic injuries (3).

However, unlike the previous studies, it was observed variable and insignificant increase in the free fraction of this drug in patients under study. Even though statistically not significant different, free fraction of phenytoin was increased in these patients and also free fraction of the drug was increased on the last day of the treatment compared to the first day. It was also noted an increase in phenytoin free fraction with reduction in plasma pH, which is yet to be studied further. We recommend that free

phenytoin concentration to be measured in the ICU patients who have risk factors for alteration in their Protein bindings (e.g. head trauma) since marked variability of free phenytoin levels was observed even in the presence of relatively stable total level.

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