**IJPSR (2021), Volume 12, Issue 4** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 March 2020; received in revised form, 25 February 2021; accepted, 22 March 2021; published 01 April 2021

# THERAPEUTIC DRUG MONITORING OF SERUM PHENYTOIN FOR EARLY POST TRAUMATIC SEIZURE PROPHYLAXIS IN TRAUMATIC BRAIN INJURY PATIENTS USING EMIT ASSAY IN A TERTIARY CARE HOSPITAL

L. Majhee [1], A. Chandra [*1], A. Prakash [2] and M. Gari [1]

Department of Pharmacology [1], Department of Neurosurgery [2], Rajendra Institute of Medical Sciences (RIMS), Ranchi - 834009, Jharkhand, India.

**Keywords:**

TDM phenytoin, TBI emit assay, India

**Correspondence to Author:**
**Dr. Anshuman Chandra**

Tutor,
Department of Pharmacology,
MMCH, House No. 27, Nilanchal Kothi Compound, Piska More, Ranchi - 834005, Jharkhand, India.

**E-mail:** dr.anshumanchandra@gmail.com

**ABSTRACT: Aims & Objective:** To monitor therapeutic drug level of serum phenytoin for early posttraumatic seizure prophylaxis in traumatic brain injury (TBI) patients using enzyme multiplied immunoassay technique (EMIT) in a tertiary care hospital. **Materials and Method:** In this observational, open-label, non-interventional, prospective study, 90 patients of mild to moderate TBI patients were recruited, and serial monitoring of total serum phenytoin level along with concomitant serum albumin was done on day 3, 5, and 7 using EMIT assay. The monitoring of phenytoin drug levels along with Glasgow coma score and any episode of posttraumatic seizures was assessed. **Results:** The most common mode of injury was road traffic accidents (61.1%). Patients with total serum phenytoin level in sub-therapeutic level on days 3, 5, and 7 were 20%, 15.6 %, 14%, respectively. The toxic range % was 0%, 1.1%, and 7% on the three respective days. **Conclusion:** This observational study concludes that serial therapeutic drug monitoring (TDM) of phenytoin in TBI patients has the advantage of optimizing the drug level within the therapeutic range and thus prevent/minimize toxicity at the earliest. Emit assay is a reliable and fast method to measure the total phenytoin level with minimum turnaround time.

**INTRODUCTION:** Traumatic brain injury is a global health problem and socioeconomic issue. TBI has been categorized as a silent epidemic, with an incidence of TBI in India is reported to be more than 15 lakhs per year. It is strongly stated that apart from reducing the incidence of TBI, prophylaxis of the short term, as well as long term clinical outcomes, has paramount importance. One of the short-term complications is posttraumatic seizures [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(4).2438-43

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(4).2438-43

After TBI, neurological damage is further enhanced due to posttraumatic seizures (PTS), which is recognized as a major complication [3]. Post Traumatic Seizure (PTS) is defined as a sudden, abnormal electrical disturbance in the brain as a sequel to traumatic brain injury (TBI). On the basis of time of occurrence following TBI, PTS may be classified as early (occurs within 1 week of injury) or late (from 1 week to many years after the TBI) [3, 4]. The incidence of early PTS is up to 25% [5].

The control of early posttraumatic seizure is of great importance because these acute insults may add secondary damage to the already insulted brain. PTS has been found to be associated with poor Glasgow outcome score (GOS). Early posttraumatic seizures are also linked with an enhanced risk factor for late posttraumatic seizures,

neuro-behavioral abnormalities, and posttraumatic epilepsy [4]. The TBI centers differ largely in their therapeutic methods for management of early PTS [4, 5]. The standard recommendation is to start phenytoin, following TBI, for 7 days for early PTS prophylaxis. Various studies have indicated that phenytoin prophylaxis decreased the incidence of early PTS from 14.2% to 3.6%. Late PTS does not benefit from phenytoin prophylaxis [3, 4]. Phenytoin is the first line anti-seizure drug for monotherapy for the seizure prophylaxis of early PTS, due to its cost-benefit and good efficacy. Phenytoin is the most studied drug and has the greatest amount of worldwide data, which encourages its use for seizure prophylaxis in early PTS [3, 8, 9]. Phenytoin, an extensively protein-bound drug (90%), fulfills prerequisites for a useful Therapeutic Drug Monitoring (TDM) with its saturable metabolism, narrow therapeutic index, nonlinear elimination kinetics, high dose variability interactions with the cytochrome P-450 isoenzyme and above all, the existing correlation between drug plasma level and drug efficacy or toxicity [7]. The benefit in seizure prophylaxis is seen with total serum phenytoin remaining within the re-commended therapeutic range (10-20 µg/ml). Without knowing the serum phenytoin levels in TBI patients, the clinician will be blindfolded, and it may be challenging to maintain the serum phenytoin level within the therapeutic range. Low drug levels will fail to adequately control the PTS, and too high levels will result in toxicity. Phenytoin has anti-seizure activity without causing general depression of the CNS. In toxic doses, it may produce excitatory signs and at lethal levels, may cause decerebrate rigidity [10].

**Aim and Objectives:** To monitor therapeutic drug level of total serum phenytoin for early posttraumatic seizure prophylaxis in traumatic brain injury (TBI) patients using Enzyme Multiplied Immuno Assay technique (EMIT) in a tertiary care hospital. To measure concomitant level of serum albumin in the TBI patients.

## MATERIALS AND METHODS:

**Ethical Consideration:** The study was approved by the Institutional Ethics Committee of the Rajendra Institute of Medical Sciences (RIMS), Ranchi, Jharkhand, India. (Memo No:-17, IAEC / IEC RIMS, RANCHI, DATED 20/02/2018).

**Place of Work:** The serum phenytoin level assessment in the TBI patients was done by EMIT assay using DIRUI® auto chemistry analyzer CST 240 in the post-graduate research laboratory, Department of Pharmacology, RIMS, Ranchi 834009.

**Study Design:** This was an observational, open-label, non-interventional, prospective study. The total duration of the study was of 12 months which was from 1st September 2018 to 31st August 2019. During this period blood sample was collected from the acute TBI patients admitted to neurosurgery department of RIMS, Ranchi. Total serum phenytoin and albumin were measured at a steady-state on 3rd, 5th, and 7th day after initiation of phenytoin therapy. Results were analyzed statistically. A sample size of 56 was considered necessary to detect statistical significance with an effect size of 0.16 at alpha 0.05 and a power of 90%. Statistical analysis was performed by the SPSS program for Windows, version [17]. 0 (SPSS, Chicago, Illinois). Continuous variables are presented as mean ± SD, and categorical variables are presented as absolute numbers and percentages. Data were checked for normality before statistical analysis. Continuous variables over time were analyzed using repeated-measures analysis of variance (ANOVA).

**Equipment Used:**

- DIRUI® auto chemistry analyzer CS- T240.
- Red top BD® Vacutainer.
- Micropipette.
- Protective gear: Glasses, gloves, and mask.
- Centrifuge.

**Reagents:**

**Phenytoin Reagents:** EMIT® 2000 Phenytoin Assay (SIEMENS Healthcare Dia-gnostics) Syva®.

**Reagent R1:** EMIT® 2000 Phenytoin Assay R1 (Antibody / Substrate) (mouse monoclonal anti-bodies reactive to phenytoin, glucose 6 phosphate, nicotinamide adenine dinucleotide.

**Reagent R2:**

- Enzyme Reagent 2 (phenytoin labelled with bacterial glucose 6 phosphate dehydrogenase G6PD).

- Emit® 2000 Phenytoin Calibrators 0, 2.5, 5, 10, 20, 40 (µg/ml).
- CS-Alkaline Detergent (Dirui® Industrial. Co. Ltd.).
- Distilled water.

**Inclusion Criteria:**

- Patient of either sex with moderate or mild head injury having Glasgow Coma Scale (GCS) score ≥9/15 admitted within 48 h of injury.
- Primary Traumatic brain injury confirmed by neurosurgery department.
- Age 15-65 years.
- Patient/ Family member who agreed for participation by written informed consent.
- Clinical or radiological features for which neurosurgery department advised the administration of phenytoin prophylaxis.

**Exclusion Criteria**

- Exposure to phenytoin at another facility or previous phenytoin use.
- Patients with a history of chronic kidney disease/nephropathy.
- Patients with a history of liver disease or coronary artery disease/heart block.
- Patients with an earlier history of status epileptics/epilepsy/history of intake of
- Any antiepileptic drugs prior to admission.
- Pregnant ladies.
- Patients with other significant injury causing hemodynamic or respiratory instability.
- Any penetrating wound in the skull.

**Procedure:** The assay method used for this study was EMIT assay.

**EMIT Assay:** Enzyme multiplied immunoassay technique

**Assay Format:** Competitive/ Homogenous assay. The study population was taken from the Department of Neurosurgery, RIMS, Ranchi. The study population consisted of 90 patients admitted with complaints of traumatic brain injury within 48 h of trauma. The patients who fulfilled inclusion criteria and were started on intravenous (I.V) phenytoin for posttraumatic seizure (PTS) prophylaxis following traumatic brain injury were included in the study. Diagnosis of TBI was confirmed by computed tomography (CT) reports and clinical case record form and examination reports available at the nursing workstation of Neurosurgery Dept. of RIMS, Ranchi. The medical records of patients were routinely checked for GCS (Glasgow Coma Scale) score and any episodes of seizures following initiation of phenytoin therapy for the period of 7 days following TBI and event duly noted. Concomitant serum albumin level was also measured on three respective days.

**RESULTS:** A total of 90 patients of the primary cause of traumatic brain injury admitted in the neurosurgery department of RIMS, Ranchi, were recruited in this study. The total serum phenytoin (trough level) was assessed for 90 patients on day 3 and day 5. There was a drop out of 30 patients on day 7, so the number of patients on day seven was 60 whose trough level (pre dose) was assessed. The demographic characteristics of the patients high-lighted that the most common age group affected by traumatic brain injury belongs to 21 to 40 years (62.3%) which is the working productive group of the community. Males (77.8%) were almost 4 folds victims of TBI as compared to females (22.2%).

**TABLE 1: TOTAL SERUM PHENYTOIN LEVEL (µg/ml) DAY 3**

| Day 3 (µg/ml) | Frequency | % |
|---|---|---|
| <10 | 18 | 20.0% |
| 10 - 20 | 72 | 80.0% |
| >20 | 0 | 0.0% |
| Total | 90 | 100% |

**TABLE 2: TOTAL SERUM PHENYTOIN LEVEL (µg/ml) DAY 5**

| Day 5 | Frequency | % |
|---|---|---|
| <10 | 14 | 15.6% |
| 10 - 20 | 75 | 83.3% |
| >20 | 1 | 1.1% |
| Total | 90 | 100% |

**TABLE 3: TOTAL SERUM PHENYTOIN LEVEL 7(µg/ml) DAY**

| Day 7 (µg/ml) | Frequency | % |
|---|---|---|
| <10 | 9 | 14.0% |
| 10 - 20 | 47 | 78.9% |
| >20 | 4 | 7.0% |
| Total | 60 | 100.0% |

**TABLE 4: MEAN VALUE OF TOTAL SERUM PHENYTOIN**

| Total Phenytoin Level (µg/ml) | N | Mean ± SD | P value |
|---|---|---|---|
| Day 3 | 60 | 11.37 ± 3.20 | P<0.001 |
| Day 5 | 60 | 12.20 ± 3.12 | |
| Day 7 | 60 | 12.84 ± 3.40 | |

**TABLE 5: INTERQUARTILE RANGE**

| Total Phenytoin Level (µml) | Mean ± SD | Min - Max | Median (IQR) |
|---|---|---|---|
| Day 3 | 11.37 ± 3.20 | 4 - 20 | 11.00 (9.80 - 12.08) |
| Day 5 | 12.20 ± 3.12 | 6 - 21 | 11.75 (10.15 - 12.92) |
| Day 7 | 12.84 ± 3.40 | 6 - 22 | 12.00 (11.00 - 14.00) |

**TABLE 6: INTER DAY COMPARISON USING ANOVA**

| Total Phenytoin(µg/Ml) | | Mean Difference | Std Error | P Value | 95% Confidence Interval For Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Day 3 | 2 | -.832* | 0.112 | <0.001 | -1.108 | -0.556 |
| | 3 | -1.464* | 0.159 | <0.001 | -1.857 | -1.071 |
| Day 5 | 1 | .832* | 0.112 | <0.001 | 0.556 | 1.108 |
| | 3 | -.632* | 0.122 | <0.001 | -0.932 | -0.332 |
| Day 7 | 1 | 1.464* | 0.159 | <0.001 | 1.071 | 1.857 |
| | 2 | .632* | 0.122 | <0.001 | 0.332 | 0.932 |

**TABLE 7: SERUM ALBUMIN LEVEL**

| S. no. Albumin (mg/dl) | N | Mean ± SD | p-value |
|---|---|---|---|
| Day 3 | 60 | 4.31 ± 0.45 | <0.002 |
| Day 5 | 60 | 4.38 ± 0.46 | |
| Day 7 | 60 | 4.41 ± 0.51 | |

**DISCUSSION:** This study was the first initiative by the Department of Pharmacology, RIMS, along with the Department of Neurosurgery, RIMS to use Therapeutic drug monitoring (TDM) to monitor the total serum concentrations of phenytoin serially in TBI patients.

This was an observational, open-label, non-interventional prospective study. EMIT assay was used to measure the total serum phenytoin levels. Phenytoin is available free of cost to inpatients of TBI admitted to the RIMS neurosurgery department. The blood samples from a patient diagnosed as a case of mild to moderate head injury were taken for measurement of trough level of total serum phenytoin. TDM of Serum phenytoin levels are included in the "High" priority group of drugs list published in WHO "Fundació Institut Català de Farmacologia." version February 18th, 2019.

It recommends the use of simple and effective methods for monitoring the phenytoin levels in setting like neuro critical units [11]. As recommended by Melissa Faye Wu and collegues in "Proceedings of Singapore Healt hcare 2013 in absence of hypo-albuminemia, total serum phenytoin is adequate for clinical use [12].

Phenytoin remains the first line anti-seizure drug for prophylaxis of early posttraumatic seizures (PTS) following TBI. The control of early posttraumatic seizure is of paramount importance because these acute insults may add secondary damage to the already insulted brain, also linked with increased incidence of late posttraumatic seizures and epilepsies [4, 5, 6, 13].

In a study done by Abraham AP and colleagues Vellore, it was found that the main limitation of their study was the measurement of a single phenytoin level as opposed to serial measurements, which would have provided a better insight into the variability of serum levels [16]. This major limitation of serial monitoring of phenytoin in early PTS was addressed in our study. As per the demographic data of our studies, the age group, sex predilection of TBI admitted to RIMS matches with that of data reported by Agrawal A and his colleagues [1, 2].

The mean age group was 35.16 ± 12.65 years, with males affected twice as females. With regards to the mechanism of injury, the statistics highlights road traffic accidents (61.1%) as a major cause followed by fall from height (27.8%) and violence being 11.1%. On serial monitoring of phenytoin over the first week after TBI, we observed that the mean total serum phenytoin tends to peak towards the higher levels at the end of the early posttraumatic period. The mean value of total serum phenytoin on day 3, day 5, and day 7 was 11.37 ± 3.20, 12.20 ± 3.12, 12.84 ± 3.40,

respectively, which was statistically significant ($p < 0.001$) **Table 1, 2, 3.** The percentage of the patient in a sub-therapeutic, therapeutic and toxic range of total serum phenytoin on day 3 was 20%, 80%, and 0%, respectively **Table 1** Loan Gh. Mohammad and colleagues reported total serum phenytoin values in percentage, using EMIT assay in three different categories as therapeutic (75%), sub-therapeutic (8.9%), and toxic (8.9), which was comparable to our study in therapeutic range [17]. Another study done by Abraham AP and colleagues reported the total serum phenytoin levels in sub-therapeutic, therapeutic, and toxic as 16.47 %, 56.47, and 27.65%, respectively [13]. Additionally, our day 5 data shows 15.6%, 83.3%, and 1.1%.

In these groups respectively **Table 4, 5**. The mean GCS on day 3, day 5, and day 7 was 12.09 ± 1.34, 12.63 ± 1.29, and 12.74 ± 1.28. Markowsky *et al.*, also demonstrated that phenytoin binding was significantly more variable with critical and convalescent patients with head injuries than healthy volunteers. One of the major causes for phenytoin toxicity in TBI patients was hypoalbuminemia, as stated. In our setting, the mean serum albumin level remained above 3.5 mg/dl.

The mean concurrent serial albumin level assessment on day 3, day 5, and day 7 was 4.31 ± 0.45, 4.38 ± 0.46 and 4.41 ± 0.51, respectively **Table 7**. Pandey M.K *et al.* assessed the serum albumin level TBI patients and observed that levels on was 3.673 ± 0.360 and Day 5 3.595 ± 0 of TBI, respectively. The study by Haltiner and colleagues had shown that the use of prophylactic phenytoin for 1 or 2 weeks reduces the incidence of early posttraumatic seizure without a significant increase in drug-related side effects.

In our study, there was no incidence of convulsive seizures or drug-related side effects in patients of TBI maintained on Phenytoin. The possibility of non-convulsive seizures could be ruled out due to the absence of an electroencephalogram in the neurosurgery department. Sahu M and colleagues in their study stated that TDM patients showed a decrease in seizure frequency by 85.44% as compared to the non-TDM group where it was only 43.81% receiving phenytoin for control of generalized tonic-clonic seizures [18]. As proposed by Melissa Faye Wu and colleagues have proposed guidelines for dosing adjustments based on phenytoin plasma concentrations have been proposed for adults in the absence of clinically significant renal or hepatic disease [12]. For plasma phenytoin concentrations less than 7 μg/ml, a dosage increase of 100 mg/day is recommended.

For plasma concentrations between 7 and 12 μg/ml, the dose may be increased by 50 mg/day, and if the plasma concentration is greater than 12 μg/ml, the dose may increase by 30 mg/day. Dosage increment, when the plasma level is above 16 μg/ml, should only be done with caution as even a small increase may result in toxicity [12]. Evaluation of Non-convulsive seizures was not possible. Continuous EEG should be done to detect non-convulsive seizure. We had to rely on clinical manifestations of the seizure activity. The actual body weight measurement in the neurocritical patient is still not possible and poses a challenge to calculate the exact dose.

**CONCLUSION:** TDM for serum phenytoin done thrice a week during the early post-traumatic period revealed that total serum phenytoin levels were found to be in sub-therapeutic range on all three days of monitoring in a significant percentage of patients and in few patients had levels in toxic range on day 5 and day 7.

This infers that TDM can be an advantage to optimize the drug level within the therapeutic range and thus prevent/minimize toxicity at the earliest. Emit assay is a reliable and fast method to measure the total phenytoin level with minimum turnaround time. Concurrent serum albumin level was found to be within the normal range. In the future, TDM could also be a screening tool to detect people who might be fast/slow metabolizers.

**ACKNOWLEDGEMENT:** I am heartily thankful to Dr. Lakhan Majhee, Associate Professor, Department of Pharmacology, RIMS, Ranchi, Jharkhand, for his valuable suggestions and guidance in this meticulous work. I extend my thanks to Dr. Anand Prakash, Associate Professor, Department of Neurosurgery, RIMS, Ranchi, for his never-ending support. Finally, I thank my son Harsh Vardhan and my wife, Dr. Swati Agrawal, for their constant love and support.

## CONFLICTS OF INTEREST: Nil

## REFERENCES:


1. WHO. Projections of mortality and burden of disease, 2002-2030 [Internet]. WHO. Available from: http:// www.who.int/healthinfo/global_burden_disease/projectios 2002.
2. Agrawal A, Munivenkatappa A and Shukla DP: Traumatic brain injury related research in India: An overview of published literature. Int J Crit Ill Inj Sci 2016; 6(2): 65-69.
3. Zafar SN, Khan AA, Ghauri AA and Shamim MS: Phenytoin versus Leviteracetam for seizure prophylaxis after brain injury -a meta analysis. BMC Neu 2012; 12: 30.
4. Ibarra M, Marta V, Fagiolino P, Mutilva F and Canale A: Total, unbound plasma and salivary phenytoin levels in critically ill patients. Journal of Epilepsy and Clinical Neurophysiology 2010; 16(2): 69-73.
5. Khattak HA, Gul N, Khan SA, Muhammad G and Khan I: Efficacy of phenytoin in prevention of early posttraumatic seizures. J Ayub Med Coll Abbot 2019; 31(2): 237-41.
6. Mee H: Pharmacological management of posttraumatic seizures in adult's current practice patterns in the UK and the Republic of Ireland. Acta Neurochirurgica Available from https://doi.org/10.1007/s00701-018-3683-9
7. Tobler A and colleagues: Free phenytoin assessment in patients measured versus calculated blood serum levels. Int J Clin Pharm 2016.
8. Candy N, Tsimiklis C, Poonnoose S and Trivedi R: The use of antiepileptic medication in early post traumatic seizure prophylaxis at a single institution.
9. Torbic H: Use of antiepileptics for seizure prophylaxis after traumatic brain injury. Am J Health- Sys Pharm-Vol 2013; 70: 1.
10. Goodman and Gilman's: The pharmacological basis of therapeutics. Edition 13th 2018.
11. Figueras A: FundacióInstitut Català de Farmacologia Version February 18th, 2019. Review of the evidence to include TDM in the Essential *in-vitro* Diagnostics List and prioritization of medicines to be monitored 2019; 1-15.
12. Wu MF: Phenytoin: A guide to therapeutic drug monitoring department of pharmacy singapore general hospital Singapore. Proceedings of Singa Health 2013; 3.
13. Head T and Greenberg MS: Handbook of neurosurgery. Ed 6th New York 2006; 632–97.
14. Brodie MJ and Kwan P: Staged approach to epilepsy management. Neurology 2002; 23: 2-8.
15. Kwon SJ, Barletta JF, Hall ST, Mangram AJ, Dzandu JK, Abdulhamid M and Zach V: Lacosamide versus phenytoin for the prevention of early post traumatic seizures. J Crit Care 2019; 50: 50-53.
16. Abraham AP, Vidyasagar A, Lakshmanan J, Nair S and Joseph M: Phenytointoxicity in patients with traumatic brain injury. Neurol India 2014; 62: 285-89.
17. Loan GM: Therapeutic drug monitoring of phenytoin needs vigilance an individualized PD response is variable. Int J of Res in Pharma & Pharm Thera 2015; 4(4): 406-10.
18. Sahu M, Kothari S, Banjare S, Sahu VB and Udenia D: Evaluate clinical impact of therapeutic drug monitoring of phenytoin monotherapy in patient of generalized tonic clonic seizures. Int A Bio Med Clin Res 2017; 3(1): 29-32.


**How to cite this article:**
Majhee L, Chandra A, Prakash A and Gari M: Therapeutic drug monitoring of serum phenytoin for early post traumatic seizure prophylaxis in traumatic brain injury patients using emit assay in a tertiary care hospital. Int J Pharm Sci & Res 2021; 12(4): 2438-43. doi: 10.13040/IJPSR.0975-8232.12(4).2438-43.