



Research paper

Correlation between serum and salivary phenytoin drug metabolite levels to phenytoin influenced gingival overgrowth in adult male subjects. A prospective cohort study



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ABSTRACT

Background: To correlate serum and salivary phenytoin drug metabolite levels in males to phenytoin influenced gingival overgrowth (PIGO).

Methods: Thirty male patients who are to start with oral phenytoin therapy were recruited. Plaque index (PI), Gingival index (GI), and Oral hygiene index (OHI) were recorded. Basic periodontal therapy was performed. Patients were followed up at 3, 6, 9 and 12 months intervals. Based on the gingival status they were divided in to two groups; Group 1 (responders) and Group 2 (non responders). Serum and Salivary samples were analyzed for the drug metabolite levels spectrophotometrically.

Results: The mean values of phenytoin drug metabolite levels in serum of group 1 and group 2 subjects were $14.5 \pm 2.6 \mu\text{g/ml}$ and $14.2 \pm 1.7 \mu\text{g/ml}$ respectively, with p value of 0.66. The corresponding mean values of phenytoin drug metabolite levels saliva were $1.42 \pm 0.34 \mu\text{g/ml}$ and $1.38 \pm 0.37 \mu\text{g/ml}$ with p value of 0.75. Correlation of phenytoin drug metabolite to PI, GI, and OHI in both the groups did not show any statistical significance. (R values ranging from -0.229 to 0.434). Correlation between the serum and salivary drug metabolites in both the responder and non responder group also did not show any statistically significant relationship.

Conclusion: No correlation between the drug concentration in either the serum or saliva can be correlated to PIGO. Whole Saliva could be a useful tool in therapeutic drug monitoring of phenytoin.

Clinical relevance: Scientific rationale: To assess and compare the drug metabolite levels in serum and saliva of a neurologic condition where therapeutic drug concentration is of key importance to minimize the side effects. **Principle finding:** Saliva could be as useful as serum in deciding the drug concentration of phenytoin. And PIGO is not related drug concentrations. **Practical implication:** Monitoring the drug dosage is of paramount importance for the success of antiepileptic therapy as well as control of its side effects. This longitudinal study confirms that saliva could be an effective alternative to serum for monitoring drug dosage of phenytoin.

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1. Introduction

Phenytoin, an anti-epileptic, was first introduced by Merritt & Putnam in 1938 for the treatment of all forms of epilepsy, except the Petit mal. In 1939, just a year after its introduction as the treatment of epilepsy, Kimbal²⁰ reported that 57% of patients treated with phenytoin developed some degrees of gingival

overgrowth.²⁵ Phenytoin is highly bound to plasma protein (90%), producing a very small free fraction. Despite the high percentage of protein binding, there can be substantial inter individual variation in free phenytoin concentrations, ranging from 9% to 25%. Therapeutic drug monitoring (TDM) is quick gaining importance as it can be effectively used to monitor drug dosages especially in neurological patients and minimize side effects of the drug. Serum has been widely used for TDM⁹; Salivary therapeutic drug monitoring offers a number of advantages over serum therapeutic drug monitoring, including lack of pain, lower cost, and wide potential acceptability by patients and physicians. It has the potential to open new approaches to treatment with strategic at-home monitoring at the time a seizure or adverse event occurs

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and to allow the collection of cohort-based, pharmacokinetic, and pharmacodynamic data for populations of persons of varying ages and with different medical conditions who require anticonvulsant medications.²⁹

Phenytoin is slowly absorbed from the gut and takes 15 days to achieve the blood plateau; it is metabolized in the liver by microsomal enzymes and excreted from urine in a conjugated form.²³ Animal studies have shown phenytoin to be concentrated in major and minor salivary gland after systemic administration of phenytoin and to be secreted in the saliva. Controversies exist in literature relating weaver salivary concentrations and serum concentrations could affect gingival overgrowth; older studies have taken in account both males and females and also many of these studies have phenytoin along with other medication.^{13,1} Importantly all of these studies had a cross sectional study design, wide age group, not adequate plaque control instruction given prior to the start of the study. Hence till date the exact pathogenesis of phenytoin influenced gingival overgrowth (PIGO) remains a debatable issue. With this background we decided to carry out a longitudinal study on male patients who are on oral phenytoin as a mono drug therapy and followed them for a year with the aim to correlate serum and salivary phenytoin levels to PIGO.

2. Materials and method

2.1. Source of data

Screening for newly diagnosed epileptic patients from August 2013 to June 2014 in the age group between 13 and 30 years, was done at Victoria Hospital and the medical outpatient department at the Bangalore Institute of Dental Sciences and Post graduate research center, Bangalore, Karnataka; India. Approval from the ethics committee of the Bangalore Institute of Dental Sciences and Post graduate research center was obtained. Inclusion criteria for the patients in the study were male patients between the age 13 to 30 years with a minimum of 20 teeth present excluding the third molars, and who are to start with oral phenytoin drug. Exclusion criteria were female patients, Patients on other drugs which are known to cause gingival overgrowth, Patients who have received any dental treatment in past six months and Patient receiving any long term medication that could interfere with Phenytoin metabolism. Apart from these smokers and alcoholics were also excluded from the study. *Demographic data:* was obtained using a structured questionnaire; patient's age, gender, socio-economical status, level of education, oral hygiene practices and habits were recorded. Thirty patients who met the inclusion and exclusion criteria and are on the same dose (100 mg tid) of oral phenytoin

Table 1

Interpretation of biochemical parameters using unpaired *t*-test between two groups.

	Responder	Non responder
Age (year)	24.07 ± 4.8	24.27 ± 5.5
Gender (male/female)	15/0	15/0
Serum Phenytoin levels (µg/ml)	14.59 ± 2.67	14.22 ± 1.77
Salivary Phenytoin levels (µg/ml)	1.42 ± 0.34	1.38 ± 0.37
Mean PI scores	2.36 ± 0.31	1.93 ± 0.71
Mean GI scores	1.18 ± 0.54	1.79 ± 0.41
Mean OHI scores	4.11 ± 0.36	2.87 ± 0.54

were explained in detail about the study and the follow up protocol; and informed consent was obtained for the same. At the end of the one year subjects were classified as responder and non responder based on the gingival status.

2.2. Clinical measurements

Thirty patients fulfilling the inclusion and exclusion criteria were recruited in to the study periodontal examination included full mouth recordings of plaque index by Turesky-Gilmore-Glickman modification of Quigley Hein Plaque Index (1970),³⁵ Gingival index (Loe and Sillness 1963)²² and Oral hygiene index (Greene and Vermilion 1960).¹² All the periodontal examination was carried out by the same examiner (Pⁱ). Degree of gingival enlargement was scored according to Bokenkamp&Bohnhorst1994². Basic periodontal therapy was performed before the patients started with the phenytoin drug and they were followed up in regular intervals of 3, 6, 9 and 12 months. Based on the gingival status the end of the one year the patients were divided into 2 groups.

Group 1: Responders – patients who showed clinical signs of gingival overgrowth.

Group 2: Non responders – patients who did not show any signs of gingival overgrowth.

2.3. Sample collection

2.3.1. Saliva collection

Unstimulated whole saliva samples were collected by drooling method. Patients were instructed to allow saliva to pool in the mouth. With head tilted forward, participants were asked to drool down to collect saliva in the cryovial. Procedure was repeated as necessary until sufficient sample was collected. 1 ml (excluding foam) was adequate for analysis. The whole saliva supernatant was frozen in liquid nitrogen, in aliquots, and subsequently stored at –20 °C until analysis.

Table 2

Pearson correlation coefficient between clinical and biochemical parameters.

Groups	Serum Phenytoin				Salivary Phenytoin			
	Parameters	GI	PI	OHI	Parameters	GI	PI	OHI
Responders	Phenytoin Serum	–0.229	–0.372	0.298	Phenytoin Saliva	0.012	0.127	0.434
	GI	1	–0.098	0.274	GI	1	–0.098	0.274
	PI	–0.098	1	–0.21	PI	–0.098	1	–0.21
	OHI	0.274	–0.21	1	OHI	0.274	–0.21	1
Non-Responders	Phenytoin Serum	–0.216	–0.243	–0.093	Phenytoin Saliva	0.545 ^a	0.197	–0.179
	GI	1	0.805 ^b	0.245	GI	1	0.805 ^b	0.245
	PI	0.805 ^b	1	0.394	PI	0.805 ^b	1	0.394
	OHI	0.245	0.394	1	OHI	0.245	0.394	1

^a Correlation is significant at the 0.05 level.

^b Correlation is significant at the 0.01 level.

Table 3
Previous studies on drug metabolites and gingival overgrowth.

s/n	Year	Study design	No of subjects	Inference
1.	Lascelles et al., 1970 ²⁴	<i>cross sectional study,</i> plasma phenytoin levels	111	A wide scatter of values was found, with over half outside the therapeutic limits of 10 to 20 µg/ml.
2.	Bochner et al., 1972 ⁴	<i>cross sectional study,</i> dosage increments on blood phenytoin levels	12	Therapeutic' range of 10–20 µg/ml., is of importance to those who use blood phenytoin levels as a guide to the adequacy of anticonvulsant therapy
3.	Gannaway and Mawer, 1981 ¹⁰	<i>cross sectional study,</i> Exploring the serum concentrations between the usually recommended range and the individual threshold level at which toxicity became just detectable	18	The same daily dose of phenytoin tended to give higher serum drug concentrations after intoxication than before.
4.	Kilpatrick and Wanwimolruk, 1984 ²¹	<i>cross sectional study,</i> To evaluate in treated epileptics the variability in phenytoin protein binding and to assess the additional clinical value of measuring the unbound concentration of the drug.	46	An inverse relationship was found between the plasma albumin concentration and the phenytoin unbound fraction
5.	Schmidt and Haenel, 1984 ³³	<i>Observational study</i> Studied the range and clinical features that influence the individual variation of the therapeutic plasma concentration of phenytoin, phenobarbital, and carbamazepine in epileptic patients on single-drug therapy.	84	Fifty-one percent of the 53 patients receiving phenytoin were completely controlled at either below or above the 10 to 20 micrograms/ml range, suggesting that individual dosage adjustment is preferably based on clinical judgment rather than numerical limits of published therapeutic ranges.
6.	Thomas and Joseph O'Donnell, 1983	<i>clinical study</i> To evaluate the putative relationships between overgrowth severity and gingival inflammation, daily phenytoin dose, age, sex and concentration of phenytoin and its major metabolite in plasma and whole saliva.	77	Positive correlations were detected between overgrowth severity and gingival inflammation, probing depths, calculus accumulation, plaque score and the measurement gingival margin to mucogingival junction. No correlation was observed between lesion severity and patient age, daily drug dosage, plasma or saliva phenytoin level, or salivary concentration of the major phenytoin metabolite.
7.	Peterson et al., 1991 ³⁰	<i>Retrospective study,</i> relationships between total and free serum concentrations of phenytoin	114	Epileptic patients have altered serum protein binding of phenytoin and display better correlations between clinical response and free, rather than total, serum levels of the drug.
8.	Thomason et al., 1992 ³⁶	<i>Observational study</i> the incidence of phenytoin-induced gingival overgrowth in a population of epileptic patients	46	No significant relationship was found between salivary phenytoin levels and the severity of the gingival overgrowth.
9.	Ball De et al., 1996 ¹	<i>Observational study</i> study and examined the relationships between plasma and saliva concentrations of phenytoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin (hphh), the major metabolite of phenytoin	36	no evidence of a relationship between phenytoin or hphh concentrations in plasma or saliva
10.	Sasaki and Maita, 1998 ³⁴	<i>Observational study</i> elucidated the involvement of bFGF (basic fibroblast growth factor) in the pathogenesis of phenytoin-induced gingival overgrowth, measured the concentration of bFGF in the serum	36	Enhanced serum bFGF level was implicated in the pathogenesis of phenytoin-induced gingival overgrowth.
11.	Birnbaum et al., 2003 ⁵	<i>Observational study</i> evaluated the intraresident variability among multiple measurements of total phenytoin serum concentrations in nursing home residents.	56	There was a considerable variability in the total phenytoin concentrations in the elderly nursing home resident and measurement of a single total phenytoin concentration should not be used to guide treatment.
12.	Guncu et al. 2006 ¹¹	<i>Observational study</i> To investigate the relationship between plasma and gingival crevicular fluid (gcf) phenytoin concentrations and the degree of gingival overgrowth in patients with similar gingival and plaque indices and also to determine the risk factors for gingival enlargement.	18	The concentration of plasma phenytoin was significantly higher in responders than non responders.

2.3.2. Blood collection

Blood samples (5 ml) from the anticubital vein was obtained and transferred into pre-heparinized coated vacutainers and immediately sent for analysis.

2.3.3. Drug analysis

Serum and Saliva levels of Phenytoin were analyzed using ultraviolet (UV) spectrophotometer. (Randox Daytona plus.) Randox laboratories, Kearneysville, WV at λ max value of 275 nm.

2.4. Statistical analysis

All the recorded clinical and biochemical parameters were statistically analyzed using SPSS software version 22 IBM.Corp.inc. Descriptive analyses including mean, standard deviation were found for each parameter in 2 groups. Comparison between two groups was performed using unpaired *t*-test. Pearson correlation test was performed for the correlation within each group.

2.5. Interpretation of biochemical parameters using unpaired *t*-test between two groups

The mean values of phenytoin drug metabolite levels in serum of group 1 and group 2 subjects were $14.5 \pm 2.6 \mu\text{g/ml}$ and $14.2 \pm 1.7 \mu\text{g/ml}$ respectively, with *p* value of 0.66. The corresponding mean values of phenytoin drug metabolite levels saliva were $1.42 \pm 0.34 \mu\text{g/ml}$ and $1.38 \pm 0.37 \mu\text{g/ml}$ with *p* value of 0.75. This difference between the two values was not statistically significant. The mean values of GI, PI and OHI in group 1 and group 2 were 1.18 ± 0.54 and 1.79 ± 0.41 ($p < 0.001$); 2.36 ± 0.31 and 1.93 ± 0.71 with ($p < 0.04$); 4.11 ± 0.36 and 2.87 ± 0.54 with ($p < 0.001$) respectively, which showed statistical significance between the groups in terms of PI and showed statistically high significance between the groups for GI and OHI Table 1.

2.6. Pearson correlation coefficient between clinical and biochemical parameters

In group 1 the correlation of phenytoin drug metabolite levels in serum and saliva with GI, PI and OHI were not statistically significant (GI: *r* value of -0.229 and 0.012 , PI: *r* -0.372 and 0.127 ; OHI: *r* 0.298 and 0.434 respectively). In group 2 correlation of phenytoin serum and phenytoin saliva levels with GI, PI and OHI were statistically not significant (GI: *r* -0.216 and 0.545 ; PI: *r* -0.243 and 0.197 ; OHI: *r* 0.093 and -0.179 respectively) Table 2.

3. Discussion

Correlation between the serum and salivary phenytoin drug metabolite levels did not show a positive correlation with PIGO after the end of one year. The mean serum drug levels were $14.59 \pm 2.67 \mu\text{g/ml}$ in the responders while the non responders were $14.22 \pm 1.77 \mu\text{g/ml}$. And the mean salivary levels were roughly one tenth of the serum levels viz $1.42 \pm 0.34 \mu\text{g/ml}$, and $1.38 \pm 0.37 \mu\text{g/ml}$ in responders and non responders respectively. Statistically significant correlation existed between plaque index and gingival index which is not of any significance in the context of the study. In our study we included only male patients with mean age of 23–29 years The explanation behind including male patients in present study is that the drug stimulates testosterone metabolism, increasing the level of 5α -dihydrotestosterone, a male sex hormone, in the overgrown tissue, which in turn, activates fibroblast growth, suggesting that the reaction may be more intense in male patients.^{1,32} Previous studies have included a wider age group and both the sexes, but phenytoin has shown to have varied rate of absorption across various age groups Evidence

suggests that there exists a serum threshold above which overgrowth occurs and that this level was lower in males.^{19,32} Hence there was a need for studies involving PIGO on male subjects which is obscure in literature (Table 3).

Incidence of gingival overgrowth can occur early within 3 months of the drug use and it may reach a state of equilibrium often within the first year of the beginning of medication. The incidence and severity is greater in the buccal surface of both upper and lower anterior teeth.²⁵ Hence in this study focuses mainly on the drug metabolite levels in the both serum and saliva The drug metabolite values in serum for both the responder and non responder groups ranged between 12 and $19 \mu\text{g/ml}$ but it was noted that the levels had no correlations with the severity of the gingival enlargement which was measured by an index.² This was in accordance to studies by,^{11,7,26} but is in contrast to other studies which reported that phenytoin concentrations in plasma or saliva are positively correlated with gingival overgrowth.^{3,6,14,27} The salivary drug metabolite levels 0.89 to 2 for both the responder and non responder groups. Interestingly 26% of the non responder patients had salivary drug metabolite levels greater than $2 \mu\text{g/ml}$. which opens debates on salivary drug metabolite role in PIGO as suggested by Babcock and Nelson 1964³. In vitro studies over three decades^{15,16,6} have stressed the importance of a distinct subpopulation of fibroblast which exists in the gingiva and its reaction to the drug metabolite could be genetically determined.^{17,18} It would seem that a certain threshold concentration of the drug or its metabolite is necessary to “activate” gingival fibroblasts, and increasing the levels of the drug above this threshold did not increase the severity of the lesion.^{8,21} This could be one possible explanation to why only certain patients subjected to the same drug have side effects. Salivary and crevicular fluid levels may provide more pertinent information on patient’s susceptibility to PIGO. It may be pertinent to note however, that dental plaque will adsorb phenytoin from the saliva.²⁸ Plaque may not only mediate its effect on overgrowth via induction but also hold phenytoin in direct contact with the tissues concerned.

The strength of this study would be its longitudinal study design, where in all the patients were followed from day 1 of start of the drug as a mono therapy to one year, reinforcing adequate plaque control methods throughout the study, age and gender bias was taken into account for; the progression of this study would be on relating to the pathogenesis of PIGO on the tissue samples obtained. Within the limitation of this study it could be concluded that neither serum nor salivary drug metabolite levels could be correlated to the degree of PIGO, it could be proposed that genetic predilection plays a crucial role in susceptibility to the side effects of the drug.

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