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Relationship between carbamazepine concentrations in serum and saliva of Thai epileptic patients

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Abstract

This study aims to examine and verify the correlation between serum and saliva carbamazepine concentrations in Thai epileptic patients. The subjects were patients aged 15-60 years old who were under treatment with carbamazepine. Carbamazepine concentrations in the blood and saliva samples were measured by a fluorescence polarization immunoassay. Blood and saliva samples of 12 patients receiving carbamazepine monotherapy were collected before and at 1, 3, 5 and 8 h after carbamazepine administration to construct a regression equation of the relationship between serum and saliva carbamazepine concentrations. Based on the results obtained, a significant linear correlation between the serum (y) and saliva (x) carbamazepine concentrations ($r = 0.929$, $p<0.001$) with a regression equation of $y = 2.402x + 2.397$ was demonstrated and being further verified by blood and saliva samples of 30 patients collected before and at 3 h after carbamazepine administration. By using the constructed regression equation, saliva carbamazepine concentrations of these 30 patients were used to estimate their respective calculated serum carbamazepine concentrations. As expected, no significant difference between the calculated and the measured carbamazepine concentrations in serum collected before carbamazepine administration in these patients. The results of the present study support the use of saliva as an alternative to serum for monitoring carbamazepine therapy.

Key Words: Carbamazepine, Serum, Saliva

Introduction

Carbamazepine is a conventional anti-epileptic drug for simple or complex partial seizure, partial with secondarily generalized seizure and generalized tonic-clonic seizures [1]. It is also effective for treating neuropathic pain and psychiatric syndromes such as bipolar disorder [1, 2]. Like other anti-epileptic drugs, therapeutic drug monitoring (TDM) is regarded as an important adjunct to treatment with carbamazepine. TDM of carbamazepine is most commonly performed using plasma or serum. Recently, there has been extensive interest in the use of saliva as an alternative matrix for TDM of anti-epileptic drugs based on its several advantages over plasma or serum [3]. In an attempt to use saliva as an alternative biological fluid for TDM, several studies investigated the relationship between carbamazepine concentrations in plasma or serum and its

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concentrations in saliva [4-21]. Carbamazepine concentrations have been determined by several methodology using high performace liquid chromatography (HPLC) [4-9], gas-liquid chromatography (GLC) [10-13], fluorescence polarization immunoassay (FPIA) [14-17], and enzyme immunoassay (EIA) [18-21]. The generally accepted therapeutic range for carbamazepine in serum (as the total form) has been estimated at 4-12 mg/L [3, 22]. Ranges reported by several groups of researchers using the same methods were rather similar, however they are somewhat different from those determined by other methods. Comparatively, the therapeutic range of saliva carbamazepine concentration in clinical setting is less established than the range in serum. A review of 17 studies revealed the saliva concentration between 0.1 to 5.5 mg/L [23] whereas an effective range of carbamazepine in saliva was suggested to be 1.4 to 3.5 mg/L by Miles et al [15]. In addition to different methodology used, the relationships between saliva and serum/plasma carbamazepine concentrations were mostly performed in Caucasians, therefore, it is unclear if the relationship holds in Asian populations. Thus, this study aimed to examine the relationship between serum and saliva carbamazepine concentrations in Thai epileptic patients using FPIA method which is the method commonly used for detection of carbamazepine. Furthermore, in order to assure the reliability of the regression equation in clinical practice; we subsequently verified the regression equation by the data of other group of patients.

Materials and Methods

Materials: The following reagents and chemicals were used in the study: TDx^R/$TDxFLx^R$ Carbamazepine reagent pack, Carbamazepine calibrators (consisted of carbamazepine solutions with concentrations of 0, 2, 4, 8, 12 and 20 μg/mL), Carbamazepine controls (consisted of carbamazepine solutions with concentrations of 3, 6 and 16 μg/mL), Dilution buffer (Abbott laboratories, IL, U.S.A.), and carbamazepine (Siam Pharmaceutical Co., Ltd., Thailand).

Subjects: The subjects were Thai epileptic patients aged between 15 to 60 years old receiving carbamazepine monotherapy (12 patients, group I) and carbamazepine monotherapy or combination therapy with other antiepileptic drugs (30 patients, group II) for at least 1 month. All were outpatients at the Department of Medicine Neurology Unit, Epilepsy Clinic, Pramongkutklao Hospital, Bangkok. The study protocol was approved by the Pramongkutklao Hospital Ethical Committee for the protection of the rights of human subjects (Approval # 1272/2549, November 28, 2006).

Study protocol: Blood and saliva samples were collected just before (0 h) and at 1, 3, 5 and 8 h after carbamazepine administration in 12 patients in group I, and just before (0 h) and 3 h after carbamazepine administration in 30 patients in group II. Samples were centrifuged at 3,000 *g* for 10 minutes at room temperature. The resulting serum and clear supernatant of saliva were stored at -80ºC until analysis. Carbamazepine concentrations in the samples were determined by FPIA using TDx® Analyzer (186I 2-96, Abbott laboratories, IL, U.S.A).

The correlation between serum and saliva carbamazepine concentrations was assessed using the data of serum and saliva carbamazepine concentrations of patients in group I. The obtained correlation equation was verified using the serum and saliva carbamazepine concentrations of patients in group II. Using the correlation equation, saliva carbamazepine concentrations of patients in group II (30 cases) were used to calculate the serum carbamazepine. Difference between the calculated serum carbamazepine in relation to its respective actual concentration (determined by FPIA) was statistically assessed.

Validation of carbamazepine assay for serum and saliva samples: Validation of the FPIA method for detection of carbamazepine in serum using the carbamazepine reagent kit has already been performed and reported by the manufacturer, thus was not performed in this study. To perform the experiment, a calibration curve was constructed using 6 concentrations (0, 2, 4, 8, 12 and 20 μg/mL) of carbamazepine standards and the calibration equation was derived. On each day, 3 control carbamazepine concentrations (3, 6 and 16 μg/mL) were used to verify the standard curve before it was used for the determination of carbamazepine concentrations in samples.

Carbamazepine concentrations in saliva specimens were measured using FPIA and the same reagent kit as used for measuring serum carbamazepine. Thus, validation of the method for detection of carbamazepine

Table 1 Accuracy, within- and between-day precision of the method using for determination of carbamazepine concentrations in saliva samples

Specimens	Carbamazepine concentrations (μg/mL)	Accuracy (%)[a]	Precision (% CV) Within-day[b]	Between-day[c]
Saliva	3	101.87 ± 1.26	3.33	4.30
	6	99.93 ± 1.55		
	16	98.95 ± 1.97		

[a] The data shown were mean ± S.D. of n = 5.
[b] The data shown were calculated from mean and S.D. of n = 5 within one day.
[c] The data shown were calculated from mean and S.D. of n = 4 (4 days).The experiments were performed in triplicate in each day.

Table 2 Summary of the demographic characteristics of the patients

Characteristics	Mean ± S.E.M. Group I (n=12)	Group II (n=30)
Age (years)	39.50 ± 2.75	34.83 ± 1.65
Body weight (kilograms)	61.67 ± 3.33	58.47 ± 1.74
Sex - male	33.33 % (4/12)	30.00 % (9/30)
- female	66.67 % (8/12)	70.00 % (21/30)
Dosage of CBZ (mg/day)*	200 - 1000	400 - 1600
Drugs combination		
	-	No (2/30)
Valproate	-	43.33 % (13/30)
Phenobarbital	-	30.00 % (9/30)
Levetiracetam	-	23.33 % (7/30)
Topiramate	-	16.67 % (5/30)
Phenytoin	-	13.33 % (4/30)
Lamotrigine	-	6.67 % (2/30)
Pharmaceutical form		
Conventional tablet	58.33 % (7/12)	20.00 % (6/30)
Controlled release tablet	41.67 % (5/12)	80.00 % (24/30)
Clinical blood chemistry	Group I & group II (n = 42)	Normal range
Blood sugar (mg%)	80.4 ± 1.2	70 - 100
BUN (mg/dL)	9.5 ± 0.5	6 - 20
Creatinine (mg/dL)	0.7 ± 0.0	0.6 - 1.2
AST (U/L)	25.2 ± 2.4	15 - 40
ALT (U/L)	20.8 ± 2.2	10 - 40
Serum total protein (g/dL)	7.6 ± 0.1	6.3 - 8.2
Serum albumin (g/dL)	4.5 ± 0.0	3.5 - 5.0
Complete blood counts		
WBC (cells/cu mm)	5,420 ± 210	5,000 – 11,000
RBC (x 10^6 cells/cu mm)	4 ± 0.08	4 - 6
Hemoglobin (g/dL)	13 ± 0.25	12 - 18
Hematocrit (%)	40 ± 0.67	35 - 51
Platelet (cells/cu mm)	243,278 ± 7782	150,000 - 400,000

*The data presented as range of n = 12 (group I) and n = 30 (group II)

in saliva was performed. The linearity of the assay was examined by spiking carbamazepine in saliva to yield 5 different concentrations of carbamazepine that were measured by FPIA using the serum carbamazepine calibration curve. The measured saliva carbamazepine concentrations were closely correlated to the actual saliva carbamazepine concentrations exhibiting a coefficient of determination (R^2) of 0.995. Accuracy of the assay was assessed by spiking carbamazepine in saliva to yield 3 concentrations (3, 6, and 16 µg/mL) of carbamazepine which were measured and the % accuracy ranged from 98.95 to 101.87% (Table 1). This is acceptable according to the criteria for biological analysis that % accuracy should be within 15% of the actual value [24]. Both within-day and between-day precision of the assay for saliva carbamazepine demonstrated a % coefficient of variation (% CV) <15% (Table 1), which is acceptable based on the criteria that % CV at each concentration should not exceed 15% [24]. The stability of carbamazepine in saliva kept at 2-8ºC for 2, 3, 4, 5, 6, 7 and 14 days ranged from 97.68 to 99.30%, based on the percentage of carbamazepine concentration after storage in relation to that prior storage.

Data analysis: The correlation between serum and saliva carbamazepine concentrations was assessed by simple linear regression and Pearson correlation analysis.

The difference between measured and calculated serum carbamazepine concentrations was analyzed by paired t-test. In all analyses, a difference was considered to be significant at $p<0.05$.

Results

All 12 patients (in group I) were prescribed carbamazepine monotherapy. Among 30 patients in group II, 2 patients (6.67 %) were given carbamazepine monotherapy while the rest received carbamazepine with other anti-epileptic drugs. Summary of demographic characteristics of patients is shown in Table 2. All serum clinical chemistry parameters of the subjects were within normal limits.

The concentrations of carbamazepine in serum (y) and saliva samples (x) of the 12 patients in group I before (0 h) and 1, 3, 5 and 8 h after carbamazepine administration were closely correlated (n = 60, r = 0.929, $p < 0.001$) and the linear regression equation, was y = 2.402x + 2.397 (Figure 1).

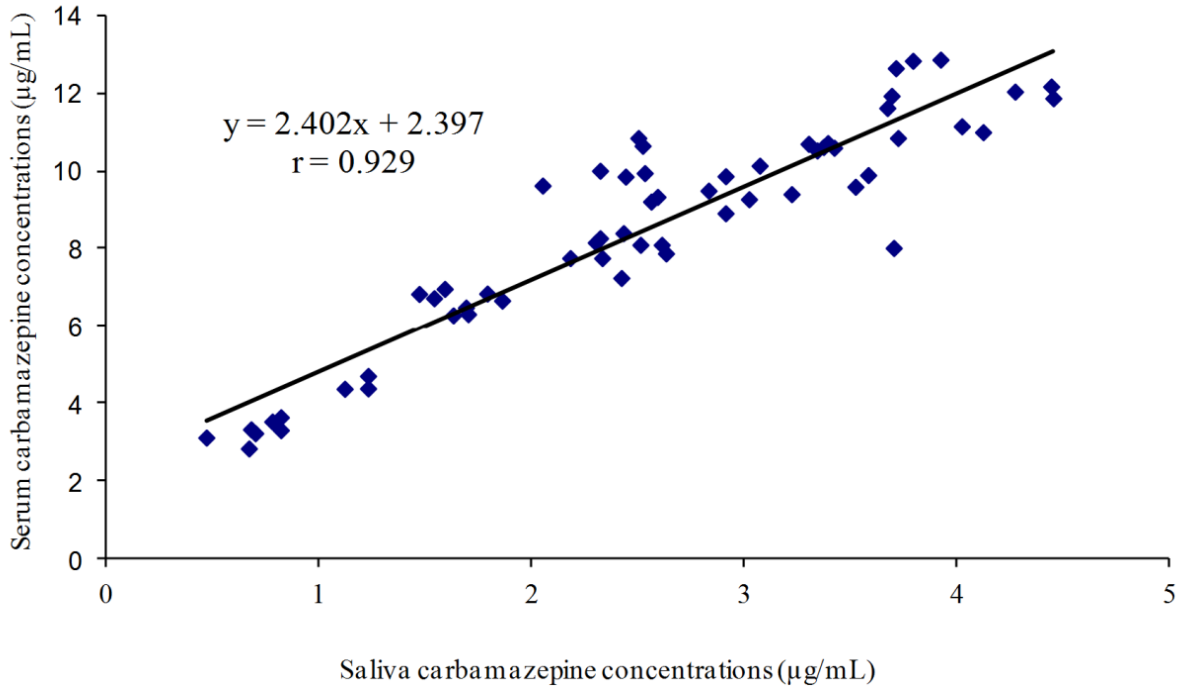

Figure 1. Correlation between serum and saliva carbamazepine concentrations determined before (0 h) and 1, 3, 5 and 8 h after carbamazepine administration (n = 60).

Reliability of the linear regression equation constructed from carbamazepine concentrations in serum and saliva samples collected from patients in group I, at all five time points after carbamazepine administration, was verified using samples collected from patients in group II. Using the regression equation (y = 2.402x + 2.397), serum carbamazepine concentrations, before (0 h) and at 3 h after carbamazepine administration, of patients in group II were calculated from their respective saliva carbamazepine concentrations. It was found that the calculated serum carbamazepine concentrations of group II patients collected at 0 h did not significantly differ (p = 0.124) from the measured serum carbamazepine concentrations (Table 3). In contrast, the calculated serum carbamazepine concentrations of group II patients collected at 3 h differed significantly (p = 0.011) from the measured serum carbamazepine concentrations (Table 4).

Discussion

The results of this study showed that serum and saliva carbamazepine concentrations were strongly and linearly correlated. The equation (y = 2.402x + 2.397) constructed from data at multiple time points (n = 60) of group I patients was verified by the data from group II patients. Verification of this equation demonstrated that the time of saliva collection for carbamazepine monitoring in practice should be at before carbamazepine administration (0 h) which is generally recommended for sample collection in routine monitoring of antiepileptic drugs that trough concentrations are measured [25]. Carbamazepine is absorbed slowly and erratically after oral administration. Peak plasma concentration of carbamazepine is usually observed at 4-8 h after oral administration, and may be delayed by as much as 24 h, particularly following a large dose administration [1]. Thus, non-completed absorption of the drug probably explains our finding that saliva collection at 3 h after carbamazepine ingestion was not as appropriate as the sample collection at the time prior to the next dose (0 h).

Therapeutic drug monitoring of carbamazepine has been most commonly done by using plasma or serum. The reference therapeutic range of carbamazepine in plasma or serum (as the total form) is 4 to 12 mg/L [3, 22]. For saliva carbamazepine concentration, Miles et al [15] has suggested an effective range of 1.4 to 3.5 mg/L, while saliva carbamazepine concentrations of less than 1.4 mg/L was unlikely to be effective for the treatment of epilepsy and concentrations greater than 3.5 mg/L was found to be associated with toxicity. In this study, serum carbamazepine concentrations found in approximately 80% of patients in group I and group II were within the therapeutic range (4 to 12 mg/L) demonstrating the saliva carbamazepine concentrations of 1.06 to 3.69 mg/L.

Carbamazepine possesses many properties suitable for salivary drug monitoring. These include small molecular size (MW = 236.27), lipophilic property and high pKa (pKa = 13.94) render it unionized within the salivary pH range [17]. Normally, protein bound drugs do not cross the membrane, thus, only the unbound fraction of the drug in serum is available for diffusion into saliva [26]. The unbound fraction of a drug is usually pharmacologically active.

Table 3 Serum carbamazepine concentrations measured before carbamazepine ingestion (0 h) and the calculated serum carbamazepine concentrations which calculated from the correlation equation: y = 2.402x + 2.397 (r = 0.929)

No. of patient	Serum carbamazepine concentrations (μg/mL)		di = measured - calculated
	Measured	Calculated	
1	9.34	9.48	-0.14
2	7.80	7.49	0.31
3	9.27	7.66	1.61
4	1.23	2.85	-1.62
5	4.96	6.05	-1.09
6	7.45	7.27	0.18
7	7.21	8.09	-0.88
8	6.98	8.47	-1.49
9	3.65	5.21	-1.56
10	8.14	8.64	-0.50
11	6.76	7.75	-0.99
12	5.21	4.77	0.44
13	8.18	8.55	-0.37
14	9.67	8.02	1.65
15	8.02	8.81	-0.79
16	1.01	2.90	-1.89
17	7.26	7.73	-0.47
18	4.03	4.94	-0.91
19	7.25	7.94	-0.69
20	6.37	5.93	0.44
21	6.28	3.98	2.30
22	3.69	4.99	1.30
23	3.03	4.15	-1.12
24	4.10	4.46	-0.36
25	7.44	7.80	-0.36
26	10.15	8.55	1.60
27	9.06	8.57	0.49
28	3.71	4.41	-0.70
29	9.72	9.19	0.53
30	6.25	8.04	-1.79
mean			-0.32
S.E.M.			0.20
			∑di = -9.49; p = 0.124

Our results suggested the use of carbamazepine concentration in whole saliva to predict total carbamazepine in serum and were even useful in term of a direct reflection of free carbamazepine concentration in serum, due to the simple passive diffusion of unbound carbamazepine through salivary gland membranes. This unbound fraction crosses most biological membranes, reaches the effector sites and should be better correlated with the effect than the total serum drug concentration. Therefore, measurement of the unbound concentration of carbamazepine seems to be better correlated with seizure control. The unbound fraction of drug in serum can be measured by performing equilibrium dialysis, ultrafiltration or ultracentrifugation prior to measurement with various techniques such as HPLC, GLC, etc. These processes are time consuming, labor intensive to perform and more costly, thus, saliva which is a natural ultrafiltrate of serum, is a superior specimen for determination of free drug concentration [22].

The mean ± S.E.M. of saliva/serum ratios of patients in group I and group II were also calculated and were shown to be 0.29 ± 0.01 and 0.29 ± 0.02, respectively. Results from this study are consistent to many previous studies reporting that the saliva/serum total carbamazepine concentration ratios ranging from 0.26-0.44 [22]. Our finding of the saliva/serum ratio of 0.29 represents the value of carbamazepine protein binding of approximately 71% which is consistent to the reported protein binding of carbamazepine of 75 % [1, 22] or 70-80 % [3].

Several methods can be used to stimulate salivation [3]. These include chewing a small square of paraffin/Teflon tape/rubber band, putting a drop of 10% citric acid on the tongue, or moving glass marble around in the mouth. Paxton et al [20] demonstrated that whole salivary and uncontaminated parotid salivary carbamazepine concentrations were similar and both were independent of volume of fluid, pH of saliva and degree of stimulation. Thus, unstimulated salivary sampling was used in the present study. By simple spitting, an adequate amount of saliva could be collected for drug level measurement. None of the patients in this study expressed any hesitancy or difficulty in spitting out saliva.

Saliva drug measurement offers an inexpensive and easy technique to measure drug concentrations. The correlation between saliva and serum carbamazepine concentrations as well as the verification of the regression equation shown in this study, indicate that measurement of carbamazepine concentrations in saliva offers a convenient and reliable method of assessing the concentration of carbamazepine in serum. Saliva therapeutic drug monitoring offers a number of advantages, including lack of pain, lower cost, and favorable acceptability by patients and physicians. In addition, stability of saliva at room temperature [16], allows it to be sent by mail, thus, at-home monitoring of carbamazepine can be performed for example immediately after a seizure or when possible toxic symptoms occur. Since the reported therapeutic range of carbamazepine in serum of 4-12 mg/L is the concentration

Table 4 Serum carbamazepine concentrations measured at 3 h after carbamazepine ingestion and the calculated serum carbamazepine concentrations which calculated from the correlation equation: y = 2.402x + 2.397 (r = 0.929)

No. of patient	Serum carbamazepine concentrations (μg/mL)		di = Measured–calculated
	Measured	Calculated	
1	9.62	9.48	0.14
2	8.35	8.38	-0.03
3	10.57	9.79	0.78
4	2.54	4.32	-1.78
5	6.06	6.34	-0.28
6	8.19	8.35	-0.16
7	7.05	8.93	-1.88
8	8.90	10.20	-1.30
9	4.26	6.48	-2.22
10	9.57	9.63	-0.06
11	8.54	8.98	-0.44
12	4.91	5.98	-1.07
13	6.74	8.83	-2.09
14	9.01	6.55	2.46
15	6.79	7.66	-0.87
16	3.21	5.18	-1.97
17	9.74	9.55	0.19
18	6.63	7.18	-0.55
19	8.64	9.58	-0.94
20	5.64	6.84	-1.20
21	6.12	5.52	0.60
22	3.27	5.21	-1.94
23	3.10	3.91	-0.81
24	5.39	6.29	-0.90
25	8.57	8.16	0.41
26	10.26	7.85	2.41
27	9.32	9.89	-0.57
28	3.91	5.38	-1.47
29	9.22	9.34	-0.12
30	6.72	8.79	-2.07
mean			-0.59
S.E.M.			0.21
			$\sum$di = -17.71; p = 0.011

of carbamazepine in the total form (unbound and bound drug), the saliva carbamazepine concentration which represents free drug in serum may be more meaningful.

In conclusion, the present study demonstrated a strong linear regression correlation between serum and saliva carbamazepine concentrations in Thai epileptic patients. Serum and saliva of 12 Thai epileptic patients with carbamazepine monotherapy were collected before carbamazepine morning dose ingestion (0 h) and at 1, 3, 5 and 8 h after carbamazepine ingestion. Concentrations of carbamazepine in saliva and serum were measured by FPIA. Linear regression correlation was shown between serum (y) and saliva (x) carbamazepine concentrations with a correlation coefficient of 0.929 ($p < 0.01$). The obtained correlation equation ($y = 2.402x + 2.397$) was verified using saliva and serum of an unrelated group of patients ($n = 30$) collecting at 0 and 3 h after carbamazepine ingestion. Calculated serum carbamazepine concentrations were obtained from adding the saliva carbamazepine concentration into the equation. No significant difference was shown between calculated serum carbamazepine concentration and the measured serum carbamazepine concentration for the sample that collected at the time before carbamazepine ingestion. This finding supports the possibility of using saliva to monitor carbamazepine therapy in clinical practice.

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None of the authors has any conflict of interest to disclose.

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