Relationship Between Serum and Saliva Theophylline Level in Thai Patients with Asthma or Copd : A Preliminary Study

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Abstract

The objective of this study was to determine a relationship between theophylline serum and saliva levels from 16 out-patients who had respiratory tract diseases (asthma and COPD) and treated with theophylline. The study was conducted in two phases at Samutsakorn hospital : 1) August to September 2000 and 2) Mid December 2000 to January 2001. The enrolled patients had averaged age (mean ± SD) of 61.44 ± 11.66. Both serum and saliva samples were analyzed for theophylline level by TDx equipment. The relationship equation of random sampling theophylline serum and saliva concentrations value from 10 out of 16 patients is C_{serum} = 1.72 C_{saliva} + 1.270 (p-value for slope = 0.001, p-value for intercept = 0.428) and correlation coefficient is 86.34% (r = 0.8634). The differences of measured theophylline serum level and predicted theophylline serum level from saliva level by the above equation from the rest 6 patients range from 9.29 to 110.30% . Mean ± SD of theophylline level in saliva and serum ratio (C_{saliva}/C_{serum}) is 0.52 ± 0.24 with 46.40% coefficient of variation (CV). This ratio is similar to the previous study in healthy volunteers. The possible factors affect this ratio are plasma protein level, affinity of drug to plasma protein and diffusion rate of drug from blood to saliva. This ratio may be useful for predicting theophylline serum level in such patients.

Introduction

Therapeutic drug monitoring (TDM) is now widely practiced. Usually, measuring drug level could be conducted from blood, urinary drug level, saliva, sweat and hair. Of these, the method based on blood level data was mostly used but there are disadvantages such as increase risk of occupational exposure of HIV infection, the fear of blood letting that occurred among patients drew back, an expensive cost, labor intensive and complex analysis in blood. In the developed country, several simplified, self- contained methods for drug concentration determination have been marketed with afford of the potential for therapeutic drug monitoring setting unaffiliated with laboratories (i.e. community pharmacies). The use of saliva may provide a simple mean for free drug concentration determination and reduce the above disadvantages for selected drugs. (Drobitch et al., 1992). Saliva was suggested as a potential substitute for serum in therapeutic monitoring for a number of medications such as theophylline, phenytoin, and carbamazepine. (Joukman JHG et al., 1981; Blanchard J.et al., 1991; Blanchard J. et al., 1992; Kark JK et al., 1994)

Over the past 30 years numerous studies have sought to describe the relationship between saliva and serum (or plasma) theophylline concentrations and to assess the reliability of predicted serum theophylline level based on salivary theophylline concentration. While many studies have found a relatively consistent relationship between saliva and serum/plasma theophylline concentration (Joukman et al., 1981; Blanchard et al., 1991; Blanchard et al., 1992; Kark et al., 1994), others have found excessive variability in the relationship (Joukman et al., 1981; Liu and Delgalado., 1999).

In 1994, a study of relationship between saliva and serum theophylline concentration was done in Thai healthy volunteer by Thongnopnua et al. This study was another that performed in asthma or COPD patients to investigate a relationship between serum and saliva theophylline concentration, and to propose and examine the correlation equation between serum and saliva concentrations in Thai asthma and COPD patients.

Method

Material

Theodur® (Astrazenica manufacturer) is a brand name of a sustained release theophylline which contains theophylline 200 mg per tablet and lot no. BB570 was used in the study. TDx (Abbott Laboratories USA. Abbott Park. IL 60064). Citric acid (B.L Hua & Co., Ltd. Lot No. S612154), theophylline (SIGMA Chemical Co. Lot No 4SHO849), TDx theophylline reagent (Abbott Laboratories USA. Abbott Park. IL 60064, Lot 65373Q100), Buffered Dilution FLX (Abbott Laboratories USA. Abbott Park. IL 60064, Lot 28952M302). All chemicals and reagents were AR grade.

Subject

This study was done in outpatients department, Samutsakorn Hospital, Samutsakorn Province, Thailand with 2 phases; 1) August to September 2000 and 2) Mid December 2000 to January 2001. The study protocol was approved by the Committee on the Protection of the Rights of Human Subject of the Hospital, and written informed consent was obtained from all patients. Men and women aged more than 20 years with asthma or COPD history were recruited into the study with the following criteria :

1. received sustained release theophylline. (Theodur®)
2. no history of receiving drugs which affect saliva secretion such as systemic anticholinergic, antidepressants, antiepileptics and chemotherapy. (Lui and Delgalado,1999)
3. no history of renal or liver disease and HIV positive.
4. no history of diseases which affect saliva secretion such as Sjogren's syndrome, congenital xerostomia, cystic fibrosis, systemic lupus erythematosus (SLE), Cushing syndrome, inflammation of salivary gland and lithiasis.
5. no caffeine and alcohol consumption on the date of collection. Patients who refused to finish the study and having adverse drug reaction to theophylline were excluded from the study.

Blood and saliva collection

Five milliliters of blood samples were collected from each subject by venipuncture in the morning before next dose after maintenance therapy at least 3 days. The samples were transferred to the 10-mL non-heparinized tubes. Saliva samples were collected by using citric acid to stimulate secretion of saliva. Five milliliters of saliva samples were transferred to 10-mL polypropylene tubes. Then both of serum and saliva samples were centrifuged at 2500 x g for 10 minutes to precipitate the particulate matter. The supernatant liquid was transferred to 1.5-mL polypropylene tube and stored at –20 degree Celsius until subsequent analysis. All of samples were analyzed by Fluorescence Polarization Immunoassay (FPIA) by TDx instrument (Abbott). The method of analysis was validated. From the literature review, theophylline in serum was stable during the storage in a blood collection tube. (Bailey et al, 1988)

Data analysis

Construction of correlation equation

When the serum/saliva theophylline concentration were obtained (C_{saliva}, C_{serum}). Ten values of theophylline in both saliva and serum concentration were randomized to construct the correlation using the simple linear regression equation (using SPSS version 9.0 and Excel 2000 programs). Relationship of 2 parameters (C_{saliva}, C_{serum}) was presented by coefficient of correlation (r) and coefficient of determination (r^2). The linear equation is represented as the following equation:

$$r = \frac{N\sum xy - \sum x \sum y}{\sqrt{(N\sum x^2 - (\sum x)^2 (N\sum y^2 - (\sum y)^2}}$$

N= number of subjects
X = Serum theophyline concentrations (mg/L)
Y = Saliva theophylline concentrations (mg/L)

Examination accuracy of correlation equation

The rest six saliva values were substituted in the constructed equation to compute the serum theophylline concentrations and compared with the measured serum theophylline levels. The percentage of difference, mean prediction error (MPE) and root mean square error (RMSE) were calculated by the following equations:

$$\%difference = \left| \frac{\text{Measured serum concentration } (c_{ob}) - \text{calculated serum concentration } (c_{cal})}{\text{Measured serum concentration}} \right| x100$$

$$MPE = \sum (C_{Ob} - C_{cal})$$

$$RMSE = \sqrt{\sum (C_{Ob} - C_{cal})^2 / N}$$

The saliva serum ratio calculation

The saliva serum ratio (C_{saliva}/C_{serum}) was calculated and presented by statistical parameters such as mean±SD.

Results

Twenty-eight patients (16 men and 12 women), with mean age of 61.44 (range 34-73 years) were enrolled in this study. Only 16 patients (10 men and 6 women) completed the study. Three patients received 200 mg of theophylline at bed time. Eleven patients received 200 mg two times a day and the rest received 200 mg in the morning. Smoking was found in 3 patients, while 6 patients used to drink the caffeine beverages and 4 of 16 patients always have an exercise. According to the classification of therapeutic range of theophylline, 6 patients (37.50%) had therapeutic serum level (10-20 μg/mL), while 10 pateints (62.5%) had subtherapuetic theophylline level (<10 μg/mL). Moreover, 7/10 of patients who had subtherapeutic theophylline level also had drug related problems. Focus on drug related problem, three patients had noncompliance, three had wrong administration and one had adverse drug reaction.

The analysis method was validated and results showed acceptable range by following the criteria of biological analysis (% CV less than 15-20%). The precision of the TDx analysis of theophylline in serum at the concentration 5, 10 and 15 μg/mL which presented by percent of coefficient of variation were 0.72, 1.16 and 1.13 respectively while these values in saliva at the same concentration were 3.03, 6.36 and 11.23, respectively. The recoveries were 92.4 ± 0.59% and 82.3 ± 0.1% for theophylline concentration in saliva and serum, respectively.

The individual serum and saliva theophylline concentration and the ratio of theophylline in saliva and serum are demonstrated in Table 1. The mean ± SD of the serum and saliva theophylline concentrations are 7.58 ± 4.49 and 3.78 ± 2.30 μg/mL, respectively and the average ratio of theophylline in saliva and serum is 0.52 ± 0.24.

To avoid the bias, the 10 random values of theophylline serum and saliva concentrations were from patients number 1, 2, 4, 6, 7, 8, 10, 11, 12 and 14. The scattered plot of the 10 individual saliva and serum theophylline is shown in Figure 1. The correlation coefficient (r) and the coefficient of determination (r^2) are 0.8634 and 0.7455, respectively and the correlation equation is presented as the following equation:

$$C_{serum} = 1.72\ C_{saliva} + 1.27 \quad (r = 0.8634,\ r^2 = 0.7455)$$

(p value of slope = 0.001, p-value of intercept = 0.428)

The determination of accuracy and precision of the equation was shown by percentage of differences between predicted serum theophylline concentrations from the correlation equation and observed serum theophylline concentrations including the Mean Prediction Error (MPE) and Root Mean Square Error (RMSE) which were presented in Table 2 .

Table 1 The individual concentration of theophylline in serum and saliva with the saliva and serum ratio.

Patient number	Serum theophylline concentration ; Cp (μg/mL)	Saliva theophylline concentration; Csal (μg/mL)	Saliva and serum ratio
1	0.56	0.17	0.30
2	15.07	6.00	0.40
3	5.64	1.59	0.28
4	7.77	1.90	0.24
5	3.68	1.60	0.43
6	4.32	1.61	0.37
7	2.84	0.97	0.34
8	12.70	7.62	0.60
9	13.72	5.00	0.36
10	6.45	4.76	0.74
11	11.17	4.84	0.43
12	10.83	5.08	0.47
13	7.25	4.63	0.64
14	3.32	3.38	1.02
15	3.63	3.7	1.02
16	12.38	7.67	0.62
Mean	**7.58**	**3.78**	**0.52**
SD	**4.49**	**2.30**	**0.24**
%CV	**59.27**	**60.93**	**46.40**

Table 2 Examination of prediction of serum theophylline concentrations from correlation equation.

Saliva theophylline concentration (mg/mL)	serum theophylline concentration (μg/mL)	Predicted serum theophylline concentration (μg/mL)	% difference	C_{ob}- C_{cal}	$(C_{ob}$- $C_{cal})^2$
1.59	5.64	4.00	28.99	1.64	2.67
1.60	3.68	4.02	9.29	-0.34	0.12
5.00	13.72	9.87	28.06	3.85	14.82
4.63	7.25	9.23	27.36	-1.98	3.93
3.70	3.63	7.63	110.30	-4.00	16.03
7.67	12.38	14.46	16.82	-2.08	4.34
			SUM	-2.93	41.92
				MPE= -0.49	RMSE = 2.64

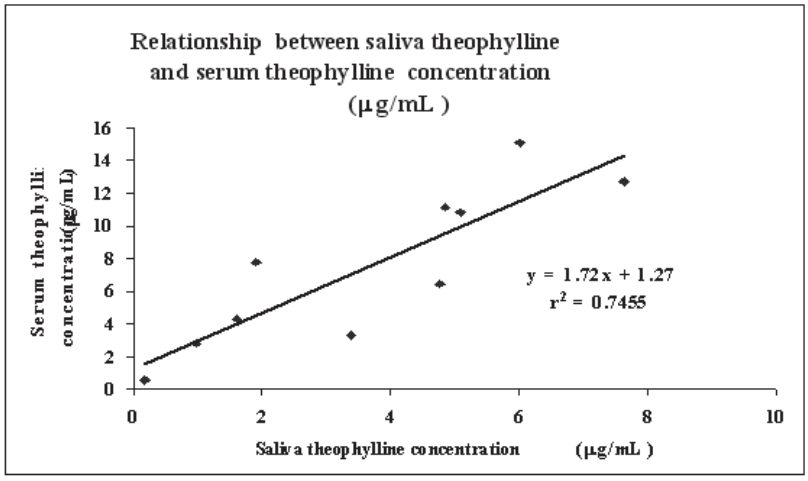

Fig. 1 Correlation between serum theophylline concentration and saliva concentration.

Discussion

According to the results, six patients (37.50%) had therapeutic theophylline serum level (10-20 μg/mL), while 10 patients (62.5%) had subtherapeutic theophylline serum level. (< 10 μg/mL). The result strongly agreed with the previous study by Tewthanom et al. (Tewthanom et al., 2000). Theophylline serum level was observed in 37 patients at Samutsakorn hospital and 35% of them had therapeutic concentration while 54.2% had subtherapeutic concentration, and 10.8% had toxic concentration. In this study, the patients who experienced subtherapeutic theophylline concentration did not manifest the asthma symptoms. It is possible because they received beta 2 agonists and steroids concurrently with theophylline. However, in these subtherapeutic level patients, about 70% (7 out of 10) experienced drug related problem including wrong administration, noncompliance, adverse drug reaction and drug interaction. In addition, coadministration with rifampicin and smoking may cause subtherapeutic theophylline

concentration. While patients who had serum level within therapeutic range, the drug related problems did not occur.

The present study was performed to investigate a relationship between serum and saliva theophylline concentration. The correlation equation was obtained from the scattered plot of the 10 randomized saliva and serum theophylline concentration. The equation is $C_{serum} = 1.72\ C_{saliva} + 1.27$ ($r^2 = 0.7455$, p value of slope = 0.001, p-value of intercept = 0.428). This equation was used to predict serum theophylline and the differences between measured and predicted theophylline concentrations were in wide range (9.29%-110.30%) with MPE and RMSE of –0.19 and 2.64, respectively. Many possible factors were proposed to affect variability of theophylline concentration and the relationship between saliva and serum theophylline concentration. One factor was the amount of plasma protein since this equation represented the relation of total serum theophylline and saliva theophylline in an unbound form. The higher in saliva theophylline concentration could occurred in patient with low plasma protein production (Frances, 1990) such as in an older patient. Additionally, pH between serum and saliva were also important factors that changed the dissociation and the distribution of theophylline . If the serum was more acidic, the saliva and serum ratio would be increased. Moreover, in patients with severe asthma or COPD, retention of excess CO_2 in serum caused acidic serum (Hendales et al., 1986) which led to the change in serum/saliva ratio. Other factors included the number of subjects enrolled in this study, ages, normal habits, and inter-individual variation. With these variabilites, it was not quite suitable to implement this equation in general practice; the result interpretation should be careful and further study are needed to confirm this result.

The mean±SD of saliva/serum ratio was also calculated as a result of 0.52±0.24. The result of this study is similar to both of the previous studies by Thongnopnua (Thongnopnua., 1994) and Siegel IA et al. (Siegel et al., 1990) which were in range of 0.48-0.6. Because this ratio range came from sufficient subjects which were wide range of ages, many variability factors was also included to analize and had statistical validity proven, so it was quite accurate.

Moreover, recent study of Thongnopnua et al. (Thongnopnua et al., 2001) was already proved by the statistical validation method that the saliva serum ratio of theophylline that calculated from healthy volunteers could be used for salivary monitoring of theophylline in Thai asthmatic patients. Therefore ,using the saliva theophylline level could be an alternative choice for monitoring theophylline level (Thongnopnua et al., 2001)

Saliva drug level could be another choice to be used in therapeutic drug monitoring as the tool for determination of drug related problem such as non-compliance as Gardain ME et al. (Gardiner et al., 1986) had found that the saliva drug level can be useful for determination of elderly compliance which useful for pharmacist as a guildeline for detection of noncompliance problem and then can be able to find the solution of this problem correctly.

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