CHARPTER IV
RESULTS

Part 1 Clinical pharmacokinetics of carbamazepine as monotherapy and in combination with classical antiepileptic drugs

Eighty five patients who used CBZ as monotherapy or coadministration with PHT, PB or VPA and their therapeutic drug monitoring data (TDM) had been recorded and available and met the inclusion criteria were included into this study. Four years retro-prospective data, August 2006 - August 2010, were collected from electronic database and medical record at the epilepsy outpatient clinic of Prasat Neurological Institute.

Demographic data

Of the 85 patients recruited, 3 patients were excluded; one patient had the PHT level lower than the analytical range, 2 patients used CBZ once daily at bedtime which CBZ levels obtained in the morning were not the trough levels. Data used for analysis included from the total of 82 patients, 79 were diagnosed to be epilepsy and 3 were neuropathic pain. Of the 79 epileptic patients, 13 had a generalized seizure and 66 had a localized seizure. Among these, 36 patients used CBZ as monotherapy, 15 patients used CBZ combination with PHT, 15 patients used CBZ combination with PB and 16 patients used CBZ combination with VPA; the details are shown in Table 12.

Table 13 presents CBZ pharmacokinetic parameters from the total patients included into the study.

Table 14 shows the comparisons of patient's characteristics and PK parameters of CBZ when categorized patients into 4 groups based on other AEDs used in combination with CBZ; CBZ monotherapy, CBZ combination with PHT (CBZ+PHT), CBZ combination with PB (CBZ+PB), and CBZ combination with VPA (CBZ+VPA). Patient's age, body weight, CBZ daily dose per body weight were not significantly different among these 4 groups, but the CBZ daily dose, CBZ level, CBZ level-to-dose ratio and CBZ clearance were significantly different among the 4 groups.

Table 12: Demographic data of patients (N=82)

Characteristic	Frequency, (mean ± SD or median)	% (range)
Number of patients	82	100
Gender		
Male	34	41.5
Female	48	58.5
Age (years)	(39.70±15.02)	(13.87–82.05)
Weight (kgs)	(61.60±12.21)	(37-104)
Indication of CBZ used		
Epilepsy	79	96
Neuropathic pain	3	4
Type of epilepsy		
Generalized seizure	13	16
Localized seizure	66	84
Combination therapy		
CBZ monotherapy	36	44
CBZ+PHT	15	18
CBZ+PB	15	18
CBZ+VPA	16	20

Table13: Pharmacokinetic parameters of CBZ from total patients included (N=82)

PK parameters (N=82)	Minimum	Maximum	Mean ± SD or Median
CBZ dose (mg/day)	200	2,000	800
(mg/kg/day)	3.33	32.33	15.45±6.53
CBZ level (mg/L)	2.10	11.90	7.50±2.43
(mcg/L/mg)	1.61	22.00	9.03±3.71
CBZ clearance (L/hr)	1.33	18.10	3.31
(L/day)	31.82	434.48	79.44
(L/kg/hr)	0.022	0.259	0.057
(L/kg/day)	0.53	6.21	1.37

Multiple comparisons of the pharmacokinetic parameters of CBZ among the 4 groups of different drug treatment in order to identify which group was different from other group were shown in details in Table 15. The result indicated that the CBZ level-to-dose ratio in CBZ monotherapy group was significantly higher than all of the other groups, and this parameter in the CBZ+PHT group was significantly lower than that observed in all of the other groups. Comparisons of the median of CBZ clearance (L/kg/hr or L/kg/day) among the 4 groups indicated that the CBZ monotherapy group had significantly lower CBZ clearance as compared to the CBZ+PHT and CBZ+PB groups, but this CBZ clearance was not significantly different from the CBZ clearance obtained from the CBZ+VPA group. At the same time, the median CBZ clearance of the CBZ+PHT group was significantly higher than that of the CBZ+VPA group.

Table 14: Comparisons of some patient's characteristics and pharmacokinetic parameters of CBZ among CBZ monotherapy and difference combination therapy groups

Parameter	Mean ± SD or Median				P-value
	CBZ (N=36)	CBZ+PHT (N=15)	CBZ+PB (N=15)	CBZ+VPA (N=16)	
Age (years) [a]	43.38 ± 14.84	34.25 ± 16.32	39.16 ± 13.37	37.02 ± 14.80	0.199
(range)	(16.53 – 82.05)	(14.13 – 64.90)	(13.87 – 61.69)	(18.35 – 65.51)	
Body weight (kgs) [b]	58.70	60.00	64.20	64.35	0.113
(range)	(40.10 – 89.00)	(37.00 – 82.00)	(47.30 – 82.00)	(43.30 – 104.00)	
CBZ dose (mg/day) [b]	800	900	1,000	1,000	0.039 *
(range)	(200 – 1,600)	(300 – 2,000)	(400 – 1,600)	(400 – 1,600)	
(mg/kg/day)	13.33	19.15	17.39	15.27	0.288
(range)	(3.33 – 29.09)	(5.19 – 27.91)	(6.23 – 30.77)	(7.08 – 32.33)	
CBZ level (mg/L) [a]	8.18 ± 2.36	5.16 ± 2.24	7.41 ± 2.16	8.24 ± 1.64	<0.001 *
(range)	(3.70 – 11.90)	(2.10 – 9.20)	(3.80 – 10.80)	(3.70 – 10.90)	
(mcg/L/mg) [b]	10.50	5.58	6.75	8.88	<0.001 *
(range)	(5.40 – 22.00)	(1.61 – 13.14)	(3.80 – 13.50)	(5.36 – 13.83)	
CBZ clearance (L/hr) [b]	2.78	5.22	4.32	3.42	<0.001 *
(range)	(1.33 – 5.40)	(2.22 – 18.10)	(2.16 – 7.68)	(2.11 – 5.44)	
(L/day)	66.67	125.37	103.70	81.86	<0.001 *
(range)	(31.82 – 129.63)	(53.26 – 434.48)	(51.85 – 184.21)	(50.60 – 130.67)	
(L/kg/hr)	0.049	0.097	0.064	0.056	0.003 *
(range)	(0.022 – 0.129)	(0.036 – 0.259)	(0.035 – 0.139)	(0.027 – 0.111)	
(L/kg/day)	1.17	2.34	1.52	1.35	0.003 *
(range)	(0.53 – 3.09)	(0.87 – 6.21)	(0.83 – 3.34)	(0.66 – 2.66)	

* Statistical significant difference ($p < 0.05$), [a] one way ANOVA test, [b] Median Test.

Table 15: Multiple comparisons of the pharmacokinetic parameters of CBZ between CBZ monotherapy and combination therapy

CBZ level (mg/L) [a]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VPA
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.667	0.029*		
	CBZ+VPA	1.00	0.001*	0.714	
	Mean±SD	8.18±2.36	5.16±2.24	7.41±2.16	8.24±1.64
CBZ level-to-dose ratio (mcg/L/mg) [b]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VP
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.008*	0.040*		
	CBZ+VPA	0.043*	0.005*	0.333	
	Median	10.50	5.58	6.75	8.88
CBZ Clearance [b]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VPA
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.008*	0.040*		
	CBZ+VPA	0.029*	0.009*	0.514	
(L/hr)	Median	2.78	5.22	4.32	3.42
(L/day)	Median	66.67	125.37	103.70	81.86
CBZ Clearance [b]	Group	CBZ	CBZ+PHT	CBZ+PB	CBZ+VP
	CBZ				
	CBZ+PHT	0.000*			
	CBZ+PB	0.036*	0.054		
	CBZ+VPA	0.341	0.002*	0.252	
(L/kg/hr)	Median	0.049	0.097	0.064	0.056
(L/kg/day)	Median	1.17	2.34	1.52	1.35

* Statistical significant differences, [a] Post Hoc test (Tukey HDS), [b] Mann-Whitney-U test.

The details about the other classical AEDs which used in combination with CBZ are shown in Table 16.

Table 16: Pharmacokinetic parameters of other AEDs used in combination with CBZ

PK parameters of other AEDs	Minimum	Maximum	Mean±SD or Median
CBZ+PHT (N=15)			
PHT dose (mg/day)	200.00	400.00	298.33 ± 69.09
PHT dose/BW (mg/kg/day)	3.33	6.67	5.01 ± 1.07
PHT level (mg/L)	4.50	32.20	15.32 ± 8.61
CBZ+PB (N=15)			
PB dose (mg/day)	30	180	120
PB dose/BW (mg/kg/day)	0.54	2.68	1.53 ± 0.73
PB level (mg/L)	7.00	32.80	13.60
CBZ+VPA (N=16)			
VPA dose (mg/day)	500	1,750	1,100
VPA dose/BW (mg/kg/day)	8.85	39.26	19.25 ± 7.68
VPA level (mg/L)	12.70	95.20	62.56 ± 20.93

Therapeutic outcome

Therapeutic outcomes were organized from the evaluations of physicians which put in the medical records. Among the 36 patients of CBZ monotherapy group, 3 patients used CBZ for neuropathic pain while 33 patients used for epilepsy. Within these 33 epileptic patients, 6 patients (18%) had uncontrolled seizure even though their CBZ levels were within the therapeutic range. A second drug had been added to 4 patients; topiramate to 3 patients and the remainder received VPA, their seizures were improved later. Because of the precipitating factors (fever, sleep late), two patients still received the same dosage of CBZ. None of the patients in CBZ monotherapy group showed sign of noticeable adverse effect (Table 17).

Among the 15 patients of CBZ+PHT combination therapy group, 4 patients (27%) still had seizure; the dosages of CBZ were increased in 2 patients and the dosages of PHT were increased in one patient, their seizures were improved later, one patient still received the same dosages of CBZ+PHT since seizure was due to precipitating factor (sleep late). There were 5 patients who had their PHT levels above the therapeutic range, 2 of them had adverse effects; nystagmus and ataxia, and their PHT dosages had been decreased (Table 17).

Among the 15 patients of CBZ+PB combination therapy group, 2 patients (13%) still had seizure; the dosage of CBZ was increased in one patient, while the rest one patient still received the same dosages of CBZ+PB since her seizure was due to precipitating factor (perimenstruation period). One patient noticed mild dizziness (Table 17).

Among the 16 patients of CBZ+VPA combination therapy group, 7 patients (44%) still had seizure; the dosage of VPA was increased in one patient and the third drug (topiramate or lamotrigine) were added in 2 patients, their seizures were improved later, the remainder 4 patients still received the same dosages of CBZ+VPA since their seizures were due to precipitating factors (sleep late, stress, perimenstruation period). One patient had mild tremor (Table 17).

Table 17: Therapeutic outcome of patients

Therapeutic levels	Efficacy		Adverse effect
	Controlled seizure	Uncontrolled seizure	
CBZ monotherapy (N=33)			
Subtherapeutic range (CBZ level < 4mg/L)	2	-	-
Therapeutic range (CBZ level 4-12 mg/L)	25	6	-
Above therapeutic range (CBZ level > 12 mg/L)	-	-	-
CBZ+PHT (N=15)			
	CBZ PHT	CBZ PHT	
Subtherapeutic range (CBZ level < 4mg/L and/or PHT <10 mg/L)	1 3	2 3	-
Therapeutic range (CBZ level 4-12 mg/L and PHT 10-20 mg/L)	3 3	1 1	-
Above therapeutic range (CBZ level > 12 mg/L and/or PHT > 20 mg/L)	- 5	- -	2
CBZ+PB (N=15)			
	CBZ PB	CBZ PB	
Subtherapeutic range (CBZ level < 4mg/L and/or PB <10 mg/L)	1 3	- -	-
Therapeutic range (CBZ level 4-12 mg/L and PB10-40 mg/L)	9 9	2 2	1
Above therapeutic range (CBZ level > 12 mg/L and/or PB > 40 mg/L)	- -	- -	-
CBZ+VPA (N=16)			
	CBZ VPA	CBZ VPA	
Subtherapeutic range (CBZ level < 4mg/L and/or VPA <50 mg/L)	1 3	- 1	1
Therapeutic range (CBZ level 4-12 mg/L and VPA 50-100 mg/L)	6 6	6 6	-
Above therapeutic range (CBZ level > 12 mg/L and/or VPA > 100 mg/L)	- -	- -	-

Part 2 Correlation between pharmacokinetic parameters of carbamazepine and other classical antiepileptic drugs when used in combination

Data from 46 patients of the 82 patients from previous part (part 1) were recruited into part 2 of this study.

Demographic data

Data included for analysis were from 46 epileptic patients, 8 had a generalized seizure and 38 had a localized seizure. There were 15 patients who used CBZ in combination with PHT, 15 patients who used CBZ in combination with PB and 16 patients who used CBZ in combination with VPA. Neither patient had serum albumin which was lower than the normal range. Demographic data of each combination therapy group is shown in table 18.

Table 18: Demographic data

Parameter	Mean ± SD or Median		
	CBZ+PHT (N=15)	CBZ+PB (N=15)	CBZ+VPA (N=16)
Age (years)	34.25 ± 16.32	39.16 ± 13.37	37.02 ± 14.80
(range)	(14.13 – 64.90)	(13.87 – 61.69)	(18.35 – 65.51)
Body weight (kgs)	61.05 ± 14.78	62.77 ± 9.98	67.09 ± 14.48
(range)	(37.00 – 82.00)	(47.30 – 82.00)	(43.30 – 104.00)
CBZ dose (mg/day)	900	1,000	1,000
(range)	(300 – 2,000)	(400 – 1,600)	(400 – 1,600)
CBZ dose/BW (mg/kg/day)	19.15	17.39	15.27
(range)	(5.19 – 27.91)	(6.23 – 30.77)	(7.08 – 32.33)
CBZ level (mg/L)	5.16 ± 2.24	7.41 ± 2.16	8.24 ± 1.64
(range)	(2.10 – 9.20)	(3.80 – 10.80)	(3.70 – 10.90)
CBZ level/dose (mcg/L/mg)	5.58	6.75	8.88
(range)	(1.61 – 13.14)	(3.80 – 13.50)	(5.36 – 13.83)
CBZ level/dose/BW (mcg/L/mg/kg)	0.12 ± 0.06	0.13 ± 0.05	0.14 ± 0.04
(range)	(0.05 – 0.23)	(0.07 – 0.24)	(0.07 – 0.22)

The details of the combination drugs which were used concurrently with CBZ are shown in Table 19. The mean daily dose per body weight of PHT from 15 patients was 5.01 ± 1.07 mg/kg/day while the mean serum level of PHT was 15.32 ± 8.61 mg/L. The mean daily dose per body weight of PB from 15 patients was 1.53 ± 0.73 mg/kg/day while the median serum level of PB was 13.60 mg/L. The mean daily dose per body weight of VPA from 16 patients was 19.25 ± 7.68 mg/kg/day and the mean serum level of VPA was 62.56 ± 20.93 mg/L.

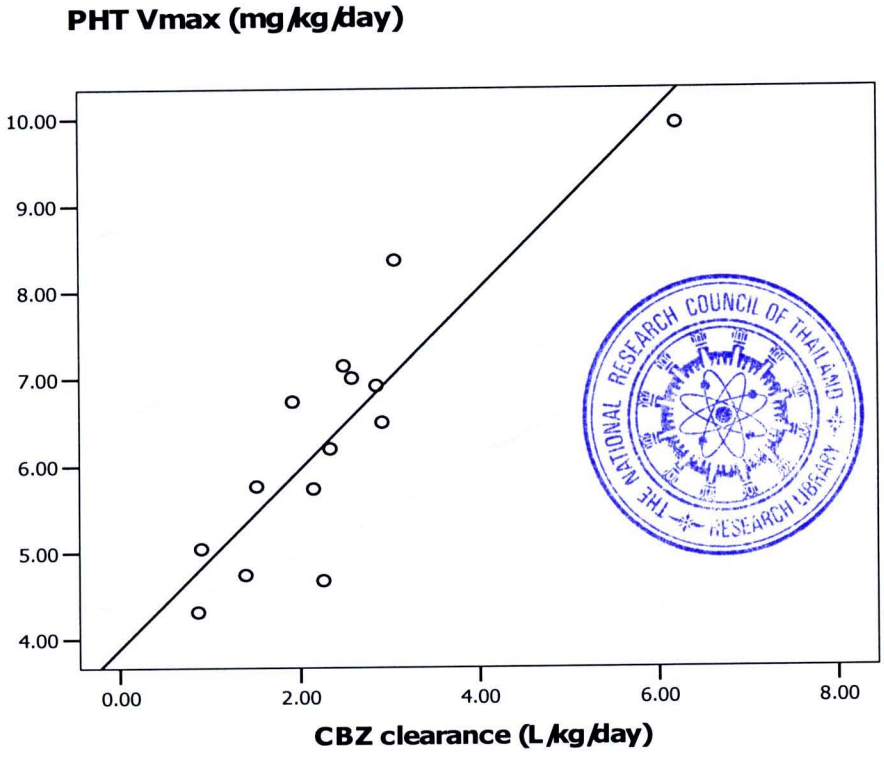
Table 19: Pharmacokinetic parameters of AEDs used in combination with CBZ

PK parameters of other AEDs	Minimum	Maximum	Mean±SD or Median
CBZ+PHT (N=15)			
PHT dose (mg/day)	200.00	400.00	298.33 ± 69.09
PHT dose/BW (mg/kg/day)	3.33	6.67	5.01 ± 1.07
PHT level (mg/L)	4.50	32.20	15.32 ± 8.61
PHT level/dose (mg/L/mg)	0.011	0.083	0.520 ± 0.025
CBZ+PB (N=15)			
PB dose (mg/day)	30	180	120
PB dose/BW (mg/kg/day)	0.54	2.68	1.53 ± 0.73
PB level (mg/L)	7.00	32.80	13.60
PB level/dose (mg/L/mg)	0.10	0.40	0.19 ± 0.07
CBZ+VPA (N=16)			
VPA dose (mg/day)	500	1,750	1,100
VPA dose/BW (mg/kg/day)	8.85	39.26	19.25 ± 7.68
VPA level (mg/L)	12.70	95.20	62.56 ± 20.93
VPA level/dose (mg/L/mg)	0.025	0.095	0.053± 0.022

Table 20 shows pharmacokinetic parameters of each patient in CBZ+PHT combination therapy group. CBZ clearance ranged from 0.87 – 6.21 L/kg/day (mean 2.45 ± 1.28 L/kg/day). PHT Vmax ranged from 4.32 – 9.93 mg/kg/day (mean 6.29 ± 1.50 mg/kg/day). The correlation between CBZ clearance and PHT Vmax was determined using regression analysis. The scatter plot of CBZ clearance versus PHT Vmax is shown in figure 6, which likely to be a simple linear correlation. The correlation between CBZ clearance and PHT Vmax was highly significant ($r = 0.817$, $p < 0.001$). There was an outlier data which was the data from patient number 2, when we excluded this data, slightly increasing in the correlation coefficient was found ($r = 0.883$, $p < 0.001$) .The regression equations between CBZ clearance and PHT Vmax are shown in Table 21.

Table 20: Pharmacokinetic parameters of individual patient in CBZ+PHT combination therapy group

Patient No	CBZ dose (mg/kg)	CBZ CL (L/day)	CBZ CL (L/kg/day)	CBZ CL (L/kg/hr)	PHT dose (mg/kg)	PHT Vmax (mg/day)	PHT Vmax (mg/kg/day)	PHT Vmax (mg/kg/hr)
1	20.90	128.61	1.92	0.080	4.48	451.24	6.73	0.28
2	10.00	200.00	3.33	0.139	3.33	317.82	5.30	0.22
3	20.00	112.90	2.26	0.094	4.00	233.73	4.67	0.19
4	19.15	134.04	2.85	0.119	6.38	325.07	6.92	0.29
5	8.11	95.45	2.58	0.107	5.41	259.10	7.00	0.29
6	26.67	112.00	2.49	0.104	6.67	321.43	7.14	0.30
7	27.91	125.37	2.92	0.121	5.81	279.22	6.49	0.27
8	25.71	434.48	6.21	0.259	5.71	695.11	9.93	0.41
9	11.14	108.95	1.52	0.063	5.57	413.71	5.76	0.24
10	6.76	159.09	2.15	0.090	5.41	424.29	5.73	0.24
11	5.19	66.67	0.87	0.036	3.90	332.33	4.32	0.18
12	11.86	53.26	0.90	0.038	4.24	297.67	5.05	0.21
13	19.23	159.09	3.06	0.127	6.25	434.78	8.36	0.35
14	9.76	114.29	1.39	0.058	3.96	388.88	4.74	0.20
15	24.69	189.19	2.34	0.097	4.01	501.67	6.19	0.26
Mean ± SD	16.47 ±7.85	146.23 ±89.16	2.45 ± 1.28	0.102 ± 0.053	5.00 ±1.07	378.40±116.76	6.29 ± 1.50	0.26 ± 0.06
Range	5.19 -27.91	53.26 -434.48	0.87 -6.21	0.036 -0.259	3.33 -6.67	233.73 -695.11	4.32 -9.93	0.18 -0.41



r = 0.883, p <0.001

Figure 6: Scatter plot of CBZ clearance (L/kg/day) versus PHT maximum rate of metabolism (mg/kg/day) (N=14).

Table 21: Regression equations show correlation between PHT maximum rate of metabolism and CBZ clearance

Regression equation	R	R Square	P- value
(N=15)			
PHT Vmax (mg/day) = 1.064 x CBZ CL (L/day) + 222.802	0.813	0.660	< 0.001
CBZ CL (L/day) = 0.621 x PHT Vmax (mg/day) – 88.595			
PHT Vmax (mg/kg/day) = 0.956 x CBZ CL (L/kg/day) + 3.945	0.817	0.668	< 0.001
CBZ CL (L/kg/day) = 0.699 x PHT Vmax (mg/kg/day) – 1.942			
(N=14)[§]			
PHT Vmax (mg/day) = 1.127 x CBZ CL (L/day) + 222.285	0.857	0.735	< 0.001
CBZ CL (L/day) = 0.652 x PHT Vmax (mg/day) – 107.266			
PHT Vmax (mg/kg/day) = 1.034 x CBZ CL (L/kg/day) + 3.889	0.883	0.780	< 0.001
CBZ CL (L/kg/day) = 0.754 x PHT Vmax (mg/kg/day) – 2.405			

[§]: excluded 1 patient (No.2 out lier data).

Table 22 shows pharmacokinetic parameters of each patient in CBZ+PB combination therapy group. CBZ clearance ranged from 0.83 – 3.34 L/kg/day (mean= 1.68 ± 0.77 L/kg/day). PB clearance ranged from 0.033 – 0.183 L/kg/day (mean= 0.084 ± 0.034 L/kg/day). The correlation between CBZ clearance and PB clearance was determined using regression analysis. There were no significant correlation between CBZ clearance versus PB clearance ($r = 0.332$, $p = 0.227$).The regression equations between CBZ clearance and PB clearance were performed and are shown in Table 23.

Table 22: Pharmacokinetic parameters of individual patient in CBZ+PB combination therapy group

Patient No	CBZ dose (mg/kg)	CBZ CL (L/day)	CBZ CL (L/kg/day)	CBZ CL (L/kg/hr)	PB dose (mg/kg)	PB CL (L/day)	PB CL (L/kg/day)	PB CL (L/kg/hr)
1	14.55	69.14	1.26	0.052	0.82	4.82	0.088	0.0037
2	16.00	84.85	1.13	0.047	0.80	5.19	0.069	0.0029
3	11.27	58.95	.83	0.035	1.69	5.10	0.072	0.0030
4	30.19	103.70	1.96	0.082	1.13	3.97	0.075	0.0031
5	30.77	169.70	3.26	0.136	1.15	5.51	0.106	0.0044
6	17.39	80.00	1.16	0.048	0.87	2.28	0.033	0.0014
7	7.14	51.85	0.93	0.039	2.68	4.12	0.074	0.0031
8	17.86	104.48	1.87	0.078	0.54	3.86	0.069	0.0029
9	6.23	56.00	0.87	0.036	1.87	4.58	0.071	0.0030
10	16.91	101.82	2.15	0.090	2.54	8.64	0.183	0.0076
11	20.00	111.36	1.59	0.066	0.86	4.03	0.058	0.0024
12	12.20	118.64	1.45	0.060	1.46	7.71	0.094	0.0039
13	17.65	103.70	1.53	0.064	1.76	7.94	0.117	0.0049
14	18.12	184.21	3.34	0.139	2.17	4.25	0.077	0.0032
15	17.67	127.27	1.87	0.078	2.65	5.51	0.081	0.0034
Mean ± SD	16.93±6.82	101.71 ± 38.45	1.68 ± 0.77	0.070 ±0.032	1.53±0.73	5.17 ± 1.73	0.084 ±0.034	0.0035 ±0.0014
Range	6.23-30.77	51.85 – 184.21	0.83 –3.34	0.035 – 0.139	0.54-2.68	2.28 – 8.64	0.033 – 0.183	0.0014 –0.0076

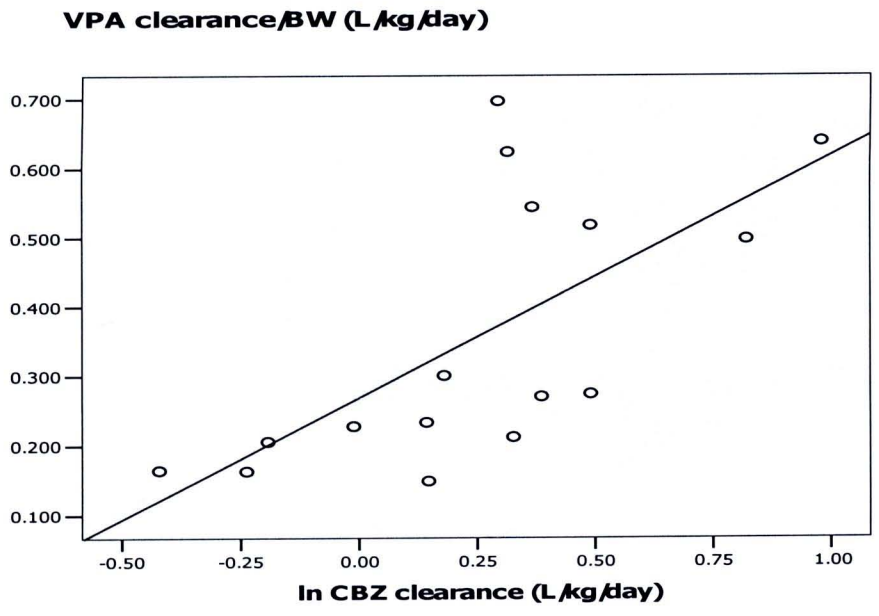
Table 23: Regression equations show correlation between PB clearance and CBZ clearance

Regression equation	R	R Square	P- value
PB CL (L/day) = 0.007 x CBZ CL (L/day) + 4.458 CBZ CL (L/day) = 3.465 x PB CL (L/day) + 83.807	0.155	0.024	0.580
PB CL(L/kg/day) = 0.014 x CBZ CL (L/kg/day) + 0.06 CBZ CL (L/kg/day) = 7.673 x PB CL (L/kg/day) + 1.032	0.332	0.110	0.227

Table 24 shows pharmacokinetic parameters of each patient in CBZ+VPA combination therapy group. CBZ clearance ranged from 0.66 – 2.66 L/kg/day (mean= 1.37 ± 0.52 L/kg/day). VPA clearance ranged from 0.149 – 0.697 L/kg/day (mean= 0.357 ± 0.193 L/kg/day). The correlation between CBZ clearance and VPA clearance was determined using regression analysis. The assumption of the linear regression was tested when we conducted the correlation equation between CBZ clearance (L/kg/day) and VPA clearance (L/kg/day). It was found that when generated the equation to predict VPA clearance from CBZ clearance, the error (observed value – predicted value) was not normally distributed, then, the CBZ clearance was transformed using log transformation (ln CBZ clearance) and the error was normally distributed. In contrary, when we generated the equation to predict CBZ clearance from VPA clearance, the error showed normal distribution. The scatter plot of ln CBZ clearance versus VPA clearance is shown in figure 7 and the scatter plot of VPA clearance versus CBZ clearance is shown in figure 8. The correlation between ln CBZ clearance and VPA clearance was moderately significant ($r = 0.661$, $p = 0.005$). The correlation between VPA clearance and CBZ clearance was moderately significant ($r = 0.642$, $p = 0.007$). The regression equations showed correlation between CBZ clearance and VPA clearance were generated and are shown in Table 25.

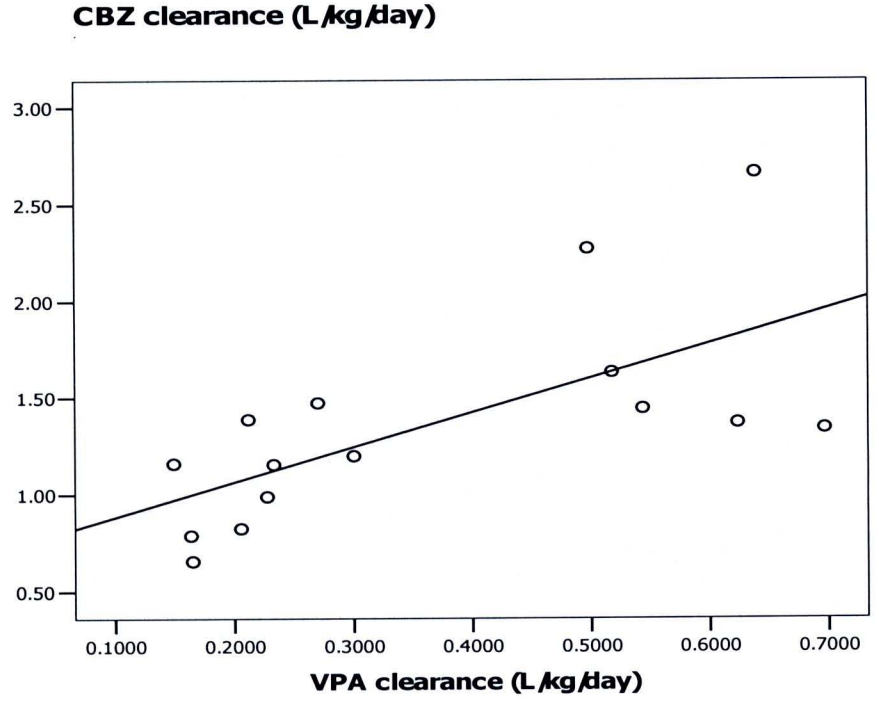
Table 24: Pharmacokinetic parameters of individual patient in CBZ+VPA combination therapy group

Patient No	CBZ dose (mg/kg)	CBZ CL (L/day)	CBZ CL (L/kg/day)	Ln CBZ CL (L/kg/day)	CBZ CL (L/kg/hr)	VPA dose (mg/kg)	VPA CL (L/day)	VPA CL (L/kg/day)	VPA CL (L/kg/hr)
1	18.92	108.89	1.47	0.39	0.061	23.65	20.00	0.270	0.0113
2	32.33	115.29	2.66	0.98	0.111	39.26	27.64	0.638	0.0266
3	14.06	78.87	1.39	0.33	0.058	17.57	12.06	0.212	0.0088
4	15.15	76.09	1.15	0.14	0.048	15.15	15.38	0.233	0.0097
5	9.30	53.16	0.82	-0.19	0.034	15.50	13.26	0.206	0.0086
6	15.38	64.22	0.99	-0.01	0.041	15.38	14.79	0.228	0.0095
7	9.09	57.73	0.66	-0.42	0.027	11.36	14.51	0.165	0.0069
8	21.92	119.15	1.63	0.49	0.068	20.55	37.78	0.518	0.0216
9	12.90	84.85	1.37	0.31	0.057	24.19	38.66	0.624	0.0260
10	13.33	71.79	1.20	0.18	0.050	16.67	18.02	0.300	0.0125
11	30.19	120.43	2.27	0.82	0.095	30.19	26.36	0.497	0.0207
12	15.87	90.91	1.44	0.37	0.060	23.81	34.25	0.544	0.0227
13	9.35	50.60	0.79	-0.24	0.033	15.58	10.50	0.164	0.0068
14	7.08	75.68	1.34	0.29	0.056	8.85	39.37	0.697	0.0290
15	15.38	120.43	1.16	0.15	0.048	11.54	15.52	0.149	0.0062
16	17.50	130.67	1.63	0.49	0.068	18.75	21.93	0.274	0.0114
Mean ± SD	16.11±7.08	88.67±26.86	1.37±0.52	0.25±0.37	0.057±0.022	19.25±7.68	22.50±10.15	0.357±0.193	0.0149±0.0080
Range	7.08-32.33	50.60–130.67	0.66 –2.66	-0.42-0.98	0.027–0.111	8.85-39.26	10.50–39.37	0.149–0.697	0.0062 – 0.0290



r = 0.661, p = 0.005

Figure 7: Scatter plot of ln CBZ clearance (L/kg/day) versus VPA clearance (L/kg/day).



r = 0.642, p = 0.007

Figure 8: Scatter plot of VPA clearance (L/kg/day) versus CBZ clearance (L/kg/day).

Table 25: Regression equations show correlation between VPA clearance and CBZ clearance

Regression equation	R	R Square	P- value
VPA CL (L/day) = 0.154 x CBZ CL (L/day) + 8.882 CBZ CL (L/day) = 1.075 x VPA CL (L/day) + 64.477	0.406	0.165	0.118
VPA CL(L/kg/day) = 0.349x ln CBZ CL (L/kg/day) + 0.269	0.661	0.437	0.005
CBZ CL (L/kg/day) = 1.732 x VPA CL (L/kg/day) + 0.754	0.642	0.412	0.007

Part 3 Effect of *CYP3A5* polymorphism on CBZ pharmacokinetics

Seventy patients who used CBZ as monotherapy or coadministration with PHT, PB or VPA and met the inclusion criteria were included into this study. A retro-prospective data, February 2010 - September 2010, were collected from electronic database and medical record at the epilepsy outpatient clinic of Prasat Neurological Institute.

Demographic data

Of the 70 patients included, 67 were diagnosed to be epilepsy and 3 were neuropathic pain. Of the 67 epileptic patients, 11 had a generalized seizure and 56 had a localized seizure. Among these, 36 patients used CBZ as monotherapy, 7 patients used CBZ combination with PHT, 11 patients used CBZ combination with PB and 16 patients used CBZ combination with VPA. The seizures of 51 patients (76%) among the 67 epileptic patients could be controlled with the current regimens. Most of the patients (83%) used folic acid as supplementation to prevent side effects; the details are shown in Table 26.

Table 26: Demographic data of patients (N=70)

Characteristic	Frequency, (mean ± SD or median)	% (range)
Number of patients	70	100
Gender		
Male	31	44
Female	39	56
Age (years)	(42.63 ± 13.83)	(16.53-82.05)
Weight (kgs)	(62.57 ± 11.76)	(40.10-104.00)
Height (cm)	(161.61 ± 8.00)	(145-185)
BMI (kg/m^2)	(23.35)	(16.50-37.53)
Indication of CBZ used		
Epilepsy	67	96
Neuropathic pain	3	4
Type of epilepsy		
Generalized seizure	11	16
Localized seizure	56	84
Seizure controlled		
Controlled	51	76
Uncontrolled	16	24
Combination therapy of AEDs		
CBZ monotherapy	36	51
CBZ+PHT	7	10
CBZ+PB	11	16
CBZ+VPA	16	23
Underlying diseases		
No other disease	47	67
Diabetes Mellitus	3	4
Dyslipidemia	13	19
Hypertension	15	21
Thalassemia	3	4
Smoking status		
Never	61	87.14
Ever smoke	1	1.43
Smoking	8	11.43

Table 26: Demographic data of patients (N=70) (continue)

Characteristic	Frequency, (mean ± SD or median)	% (range)
Alcohol consumption		
Never	67	96
Ever drink	1	1
Drinking	2	3
Adverse effect		
No adverse effect	66	94.3
Tremor	1	1.4
Dizziness	2	2.9
Ataxia	1	1.4
AST (IU/L), N= 29	(21.00)	(9-64)
ALT (IU/L), N= 29	(15.00)	(3-54)
Serum albumin (g/dL), N= 32	(4.10)	(2.5-4.7)
Serum creatinine (mg/dL), N= 27	(0.90)	(0.50-1.40)
Co-medications		
Folic acid	58	83
Simvastatin	10	14
Calcium carbonate	8	11
Enalapril	7	10
HCTZ	6	9
Vitamin B complex	6	9
Multivitamin	5	7
Clobazam	4	6
Atenolol	4	6
Amlodipine	3	4
Manidipine	3	4
Rosuvastatin	3	4
Metformin	2	3
Ezetrimide	1	1
Atorvastatin	1	1
Clopidogrel	1	1
Aspirin	1	1
Glibenclamide	1	1

Table 27 presents CBZ pharmacokinetic parameters from the total patients included into the study. All patients included into this part were the same patients that included into part 1 except for the twelve patients who lack of the genetic data were excluded. The pharmacokinetic parameters of CBZ from total patients in this part were closed to previous part.

Table 27: Pharmacokinetic parameters of CBZ from total patients included (N=70)

PK parameters (N=70)	Minimum	Maximum	Mean ± SD or Median
CBZ dose (mg/day)	200	2,000	800
(mg/kg/day)	3.33	32.33	14.59 ± 5.90
CBZ level (mg/L)	2.10	11.90	7.74 ± 2.39
(mcg/L/mg)	2.63	22.00	9.51 ± 3.67
CBZ clearance (L/hr)	1.33	11.11	3.15
(L/day)	31.82	266.67	75.68
(L/kg/hr)	0.022	0.185	0.054
(L/kg/day)	0.53	4.44	1.29

Population allelic frequencies

Genotyping of *CYP3A5* was obtained from 70 patients, 36 patients used CBZ as monotherapy, 7 patients used CBZ in combination with PHT, 11 patients used CBZ in combination with PB and 16 patients used CBZ in combination with VPA. When characterized the patients into 3 groups by *CYP3A5* genotyping, there were 8 patients (11%) with homozygous **1/*1*, 28 patients (40%) with heterozygous **1/*3* and 34 patients (49%) with homozygous **3/*3*. The allele frequency of *CYP3A5*1* was 31% and *CYP3A5*3* was 69%. The details were shown in Table 28.

Table 28: Prevalence of *CYP3A5* genotype

(70 patients x 2 alleles)				Genotypes	Observed N=70	%	Predicted (HWE)
Alleles	N=140	%	95%CI				
***1**	44	31	23.5-38.5	***1/*1**	8	11	7
				***1/*3**	28	40	30
***3**	96	69	61.5-76.5	***3/*3**	34	49	33
					Chi-square=0.306, p=0.858		

Allelic frequencies of *CYP3A5* genotypes were in Hardy-Weinberg Equilibrium (HWE), p =0.858. The calculation if allelic frequencies were in HWE:

The number of the *1 allele = (8 x 2) + (28 x 1) = 44 alleles

The number of the *3 allele = (34 x 2) + (28 x 1) = 96 alleles

The frequency of the *1 allele = p = 44 / (44 + 96) = 0.31

The frequency of the *3 allele = q = 96 / (44 + 96) = 0.69

The proportion of expected *1/*1, *1/*3 and *3/*3 genotypes could be predicted from HWE: $p+q = 1$ and $(p + q)^2 = 1$ or $p^2 + 2pq + q^2 = 1$

$p^2 = 0.31 \times 0.31 = 0.0961$

$2pq = 2 \times 0.31 \times 0.69 = 0.4278$

$q^2 = 0.69 \times 0.69 = 0.4761$

The total number of patients included to this study was 70

Expected number of *1/*1 = $0.0961 \times 70 = 6.73 \approx 7$

Expected number of *1/*3 = $0.4278 \times 70 = 29.95 \approx 30$

Expected number of *3/*3 = $0.4761 \times 70 = 33.32 \approx 33$

The observed number of *1/*1 = 8

The observed number of *1/*3 = 28

The observed number of *3/*3 = 34

Chi-square =0.306, p=0.858

Therefore, could not reject the null hypothesis that the population is in HWE.

Effect of CYP3A5 polymorphism on CBZ pharmacokinetics

Seventy patients were categorized by *CYP3A5* genotypes into 3 groups; *CYP3A5*1/*1*, *CYP3A5**1/*3, and *CYP3A5*3/*3*. Patient's age, body weight, BMI, the frequency of patients when categorized by gender and coadministration drugs were not significantly different among these 3 groups. The details about demographic data of patients when categorized by *CYP3A5* genotypes are shown in Table 29.

Table 29: Demographic characteristics of patients when categorized patients into 3 groups based on *CYP3A5* genotypes

Demographic data	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	p-value
No. of patients	8	28	34	
Gender (male/female) [a]	3/5	12/16	16/18	0.602
Age (yr) [b]	50.96±20.61	38.97±11.47	43.68±13.16	0.078
(range)	(16.53-82.05)	(18.35-64.90)	(17.81-69.77)	
Body weight (kg) [b]	66.48±12.51	58.56±9.04	64.95±12.89	0.061
(range)	(52.00-88.00)	(40.10-77.00)	(43.30-104.00)	
BMI (kg/m^2) [b]	24.01±2.26	22.73±2.85	24.93±4.79	0.093
(range)	(21.37-27.85)	(17.26-29.34)	(16.50-37.53)	
Coadministration drugs [a]				
CBZ monotherapy	7	14	15	0.061
CBZ+PHT	0	3	4	0.897
CBZ+PB	0	4	7	0.521
CBZ+VPA	1	7	8	0.660

[a] Chi-square test, [b] One-way ANOVA.

Table 30 shows the comparisons of patient's PK parameters of CBZ when categorized patients into 3 groups based on their *CYP3A5* genotypes. CBZ dose, CBZ level and CBZ clearance were not significantly different among these 3 groups.

Table 30: Pharmacokinetic parameters of CBZ when categorized patients into 3 groups based on *CYP3A5* genotypes

Parameter	Mean±SD or Median			p-value
	*CYP3A5*1/*1* (N=8)	*CYP3A5*1/*3* (N=28)	*CYP3A5*3/*3* (N=34)	
CBZ dose (mg/day) [a]	800	800	800	0.366
(range)	(400-800)	(400-1,600)	(200-2,000)	
(mg/kg/day) [b]	11.16±2.96	15.63±6.25	14.53±5.94	0.168
(range)	(6.67-15.38)	(5.19-30.19)	(3.33-32.33)	
CBZ level (mg/L) [a]	8.40	8.35	8.00	0.982
(range)	(3.70-9.70)	(2.10-11.80)	(2.20-11.90)	
(mcg/L/mg)	10.50	9.22	9.25	0.512
(range)	(6.17-21.50)	(2.63-18.60)	(3.70-22.00)	
CBZ clearance (L/hr) [a]	2.78	3.16	3.15	0.512
(range)	(1.36-4.73)	(1.57-11.11)	(1.33-7.88)	
(L/day)	66.71	75.88	75.68	0.518
(range)	(32.56-113.51)	(37.63-266.67)	(31.82-189.19)	
(L/kg/hr)	0.043	0.055	0.054	0.220
(range)	(0.023-0.074)	(0.028-0.185)	(0.022-0.111)	
(L/kg/day)	1.03	1.33	1.30	0.223
(range)	(0.54-1.78)	(0.68-4.44)	(0.53-2.66)	

[a] Kruskal-Wallis H test, [b] One-way ANOVA.

When we categorized patients into 2 groups based on *CYP3A5* genotypes; the first group was *CYP3A5*1/*1* and *CYP3A5*1/*3*, and the second group was *CYP3A5*3/*3*. Patient's age, body weight, the frequency of patients based on gender and coadministration drugs were not significantly different between these 2 groups, while the mean BMI in the *CYP3A5*1/*1* and *CYP3A5*1/*3* group was significantly (p=0.047) lower than that of the *CYP3A5*3/*3* group. The details about demographic data of patients when categorized by *CYP3A5* genotypes are shown in Table 31.

Table 31: Demographic characteristics of patients when categorized patients into 2 groups based on *CYP3A5* genotypes

Demographic data	*CYP3A5*1/*1* and **1/*3*	*CYP3A5*3/*3*	p-value
No. of patients	36	34	
Gender (male/female) [a]	15/21	16/18	0.650
Age (yr) [b]	41.63±14.56	43.68±13.16	0.541
(range)	(16.53-82.05)	(17.81-69.77)	
Body weight (kg) [b]	60.32±10.27	64.95±12.89	0.100
(range)	(40.10-88.00)	(43.30-104.00)	
BMI (kg/m^2) [b]	23.01±2.75	24.93±4.79	0.047
(range)	(17.26-29.34)	(16.50-37.53)	
Coadministration drugs [a]			
CBZ monotherapy	21	15	0.234
CBZ+PHT	3	4	0.706
CBZ+PB	4	7	0.276
CBZ+VPA	8	8	0.896

[a] Chi-square test, [b] independent t-test.

Table 32 shows the comparisons of patient's PK parameters of CBZ when categorized patients into 2 groups based on their *CYP3A5* genotypes. CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups.

Table 32: Pharmacokinetic parameters of CBZ when categorized patients into 2 groups based on *CYP3A5* genotypes

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and **1/*3* (N=36)	*CYP3A5*3/*3* (N=34)	
CBZ dose (mg/day) [a]	800	800	0.516
(range)	(400-1,600)	(200-2,000)	
(mg/kg/day) [b]	14.64±5.95	14.53±5.94	0.940
(range)	(5.19-30.19)	(3.33-32.33)	
CBZ level (mg/L) [a]	8.35	8.00	0.991
(range)	(2.10-11.80)	(2.20-11.90)	
(mcg/L/mg) [b]	9.74±3.91	9.27±3.44	0.599
(range)	(2.63-21.50)	(3.70-22.00)	
CBZ clearance (L/hr) [a]	3.04	3.15	0.634
(range)	(1.36-11.11)	(1.33-7.88)	
(L/day) [a]	72.84	75.68	0.634
(range)	(32.56-266.67)	(31.82-189.19)	
(L/kg/hr) [a]	0.054	0.054	0.991
(range)	(0.023-0.185)	(0.022-0.111)	
(L/kg/day) [a]	1.29	1.30	1.00
(range)	(0.54-4.44)	(0.53-2.66)	

[a] Mann-Whitney U test, [b] independent t-test.

The patient's characteristics and PK parameters of CBZ between different *CYP3A5* genotypes were further compared through sub groups analysis based on the coadministration drugs; CBZ monotherapy, CBZ in combination with PHT, CBZ in combination with PB, CBZ in combination with VPA and CBZ in combination with enzyme inducing AED (CBZ in combination with PHT or PB). The details were shown in Table 33-37.

Among the 36 patients of CBZ monotherapy group, there were 21 patients (58%) who are *CYP3A5*1/*1* and **1/*3*, and 15 patients (42%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 33A).

Among the 36 patients of CBZ monotherapy group, there were 7 patients (19%) who are *CYP3A5*1/*1*, and 29 patients (81%) who are *CYP3A5*1/*3* and **3/*3*. Patient's body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes, while the mean of age in patients who are *CYP3A5*1/*1* (54.33±19.73 yrs) was significantly higher (p=0.028) than the mean of age in patients who are *CYP3A5*1/*3* and **3/*3* (40.76±12.46 yrs) (Table 33B).

Among the 7 patients of CBZ in combination with PHT group, there were 3 patients (43%) who are *CYP3A5*1/*3*, and 4 patients (57%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 34). Figure 9 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 10 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

Table 33A: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ monotherapy group between *CYP3A5*1/*1* and **1/*3* VS *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and **1/*3* (N=21)	*CYP3A5*3/*3* (N=15)	
Age (yr) [a]	43.47±15.62	43.30±14.25	0.974
(range)	(16.53-82.05)	(17.81-69.77)	
Body weight (kg) [a]	57.12±9.36	61.37±11.33	0.227
(range)	(40.10-80.50)	(45.00-89.00)	
BMI (kg/m^2) [a]	22.17±2.57	23.33±3.44	0.253
(range)	(17.26-27.85)	(16.73-30.80)	
CBZ dose (mg/day) [b]	800	800	0.300
(range)	(400-1,600)	(200-1,400)	
(mg/kg/day) [a]	13.98±5.72	14.29±5.46	0.871
(range)	(6.67-29.09)	(3.33-23.53)	
CBZ level (mg/L) [a]	8.02±2.29	8.39±2.51	0.645
(range)	(3.70-11.80)	(4.40-11.90)	
(mcg/L/mg) [a]	11.06±3.92	10.61±3.65	0.727
(mcg/L/mg) [b]	10.75	9.92	0.619
(range)	(5.40-21.50)	(6.75-22.00)	
CBZ clearance (L/hr) [a]	2.96±1.06	2.97±0.76	0.972
(range)	(1.36-5.40)	(1.33-4.32)	
(L/day) [a]	71.06±25.47	71.32±18.20	0.973
(range)	(32.56-129.63)	(31.82-103.70)	
(L/kg/hr) [a]	0.053±0.023	0.049±0.013	0.552
(range)	(0.023-0.129)	(0.022-0.071)	
(L/kg/day) [a]	1.28±0.55	1.18±0.32	0.552
(range)	(0.54-3.09)	(0.53-1.70)	

[a] independent t-test, [b] Mann-Whitney U test.

Table 33B: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ monotherapy group between *CYP3A5*1/*1* VS *CYP3A5*1/*3* and **3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* (N=7)	*CYP3A5*1/*3* and **3/*3* (N=29)	
Age (yr) [a]	54.33±19.73	40.76±12.46	0.028*
(range)	(16.53-82.05)	(17.81-69.77)	
Body weight (kg) [a]	63.40±9.71	57.80±10.29	0.201
(range)	(52.00-80.50)	(40.10-89.00)	
BMI (kg/m^2) [a]	23.76±2.33	22.38±3.08	0.276
(range)	(21.37-27.85)	(16.73-30.80)	
CBZ dose (mg/day) [b]	800	800	0.360
(range)	(400-800)	(200-1,600)	
(mg/kg/day) [a]	11.46±3.07	14.74±5.84	0.161
(range)	(6.67-15.38)	(3.33-29.09)	
CBZ level (mg/L) [b]	8.20	8.70	0.263
(range)	(3.70-9.00)	(3.70-11.90)	
(mcg/L/mg) [b]	10.25	10.50	0.749
(mcg/L/mg) [a]	11.13±4.90	10.81±3.54	0.844
(range)	(6.17-21.50)	(5.40-22.00)	
CBZ clearance (L/hr) [a]	2.97±1.03	2.96±0.93	0.983
(range)	(1.36-4.73)	(1.33-5.40)	
(L/day) [a]	71.32±24.75	71.13±22.31	0.985
(range)	(32.56-113.51)	(31.82-129.63)	
(L/kg/hr) [b]	0.046	0.049	0.603
(L/kg/hr) [a]	0.048±0.017	0.053±0.020	0.543
(range)	(0.023-0.074)	(0.022-0.129)	
(L/kg/day) [b]	1.11	1.17	0.617
(range)	(0.54-1.78)	(0.53-3.09)	

* Statistical significant difference, [a] independent t-test, [b] Mann-Whitney U test.

Table 34: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+PHT group between *CYP3A5 *1/*3* and *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=3)	*CYP3A5*3/*3* (N=4)	
Age (yr) [a]	48.98±15.87	45.36±9.75	0.721
(range)	(33.16-64.90)	(35.09-57.97)	
Body weight (kg) [a]	68.00±8.54	74.00±10.61	0.461
(range)	(60.00-77.00)	(59.00-82.00)	
BMI (kg/m^2) [a]	26.10±2.81	29.47±5.01	0.348
(range)	(24.31-29.34)	(22.48-34.13)	
CBZ dose (mg/day) [a]	866.67±503.32	1,000±678.23	0.788
(range)	(400-1,400)	(500-2,000)	
(mg/kg/day) [a]	13.14±7.86	13.27±7.90	0.984
(range)	(5.19-20.90)	(6.76-24.69)	
CBZ level (mg/L) [a]	4.64±2.79	5.92±3.05	0.592
(mg/L) [b]	4.20	6.15	0.480
(range)	(2.10-7.62)	(2.20-9.20)	
(mcg/L/mg) [a]	6.19±3.99	6.84±4.32	0.846
(mcg/L/mg) [b]	5.44	5.26	0.724
(range)	(2.63-10.50)	(3.70-13.14)	
CBZ clearance (L/hr) [a]	6.42±4.26	5.37±2.46	0.696
(L/hr) [b]	5.36	5.70	0.724
(range)	(2.78-11.11)	(2.22-7.88)	
(L/day) [a]	153.98±102.39	128.96±59.11	0.697
(L/day) [b]	128.61	136.69	0.724
(range)	(66.67-266.67)	(53.26-189.19)	

[a] independent t-test, [b] Mann-Whitney U test.

Table 34: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+PHT group between *CYP3A5 *1/*3* and *CYP3A5*3/*3* (continue)

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=3)	*CYP3A5*3/*3* (N=4)	
CBZ clearance (L/kg/hr) [a]	0.100±0.077	0.071±0.028	0.497
(L/kg/hr) [b]	0.080	0.074	1.00
(range)	(0.036-0.185)	(0.038-0.097)	
(L/kg/day) [a]	2.41±1.84	1.70±0.67	0.495
(L/kg/day) [b]	1.92	1.77	1.00
(range)	(0.87-4.44)	(0.90-2.34)	

[a] independent t-test, [b] Mann-Whitney U test.

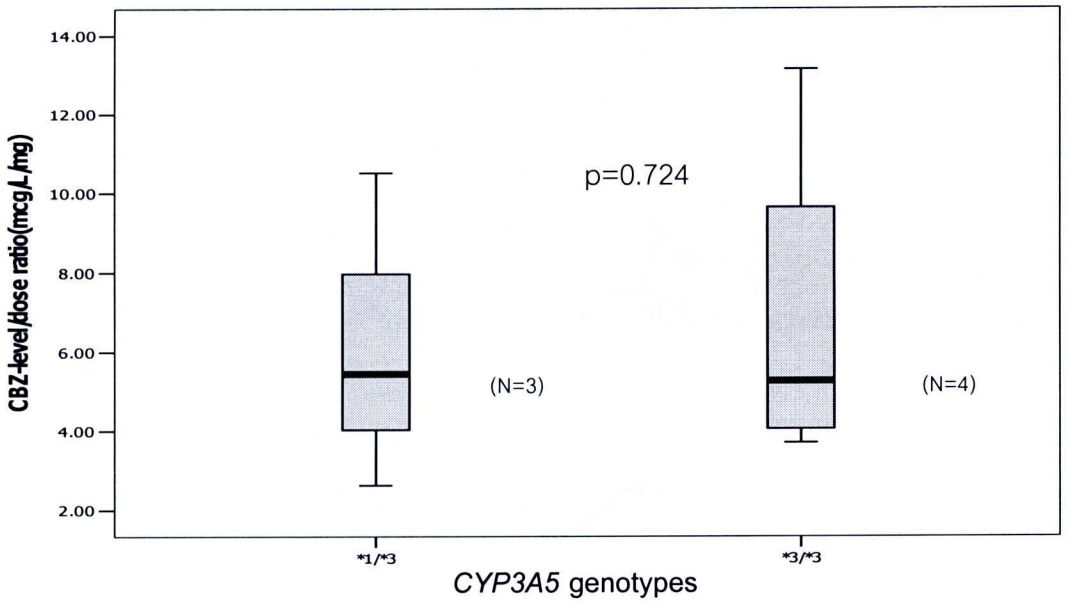


Figure 9: Box and whisker plot of the median CBZ level (mcg/L/mg) between different genotypes in CBZ+PHT group (N=7).

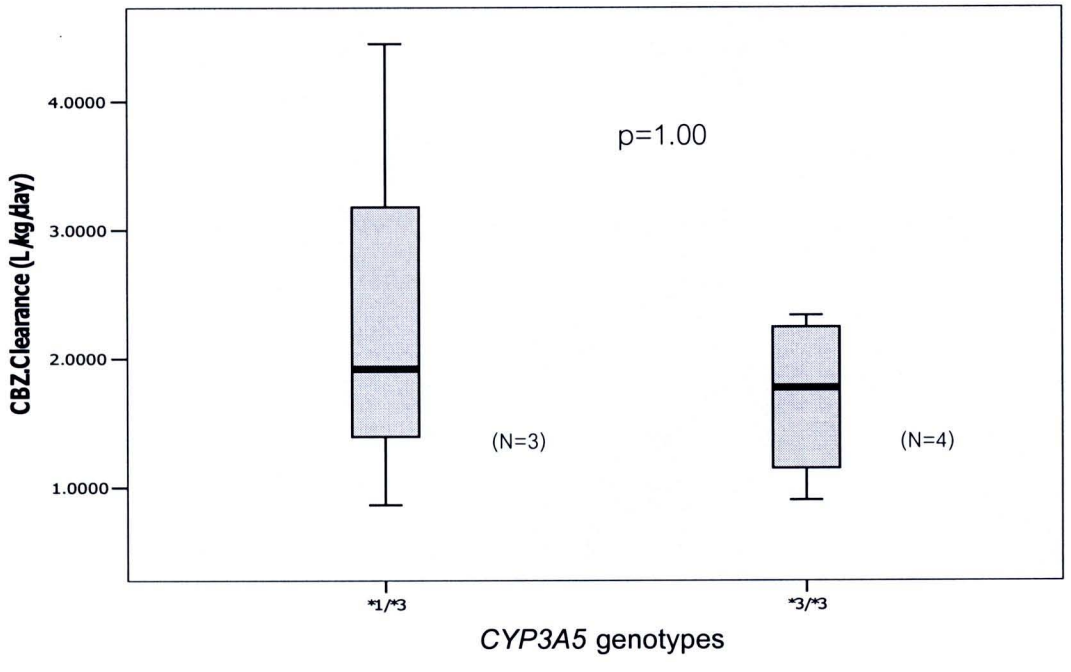


Figure 10: Box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes in CBZ+PHT group (N=7).

Among the 11 patients of CBZ concurrently used with PB group, there were 4 patients (36%) who are *CYP3A5*1/*3*, and 7 patients (64%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 35). Figure 11 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 12 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

Among the 16 patients of CBZ in combination with VPA group, there were 8 patients (50%) who are *CYP3A5*1/*1* and **1/*3*, and 8 patients (50%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 36). Figure 13 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 14 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

Among the 18 patients of CBZ in combination with enzyme inducing AED (CBZ+PHT and CBZ+PB) group, there were 7 patients (39%) who are *CYP3A5*1/*3*, and 11 patients (61%) who are *CYP3A5*3/*3*. Patient's age, body weight, BMI, CBZ dose, CBZ level and CBZ clearance were not significantly different between these 2 groups of different genotypes (Table 37). Figure 15 shows box and whisker plot of the median CBZ level (mcg/L/mg) and Figure 16 shows box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes.

Table 35: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+PB group between *CYP3A5 *1/*3* and *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=4)	*CYP3A5*3/*3* (N=7)	
Age (yr) [a]	44.91±6.76	45.88±9.19	0.859
(range)	(39.26-53.82)	(32.58-61.69)	
Body weight (kg) [a]	59.85±10.45	63.89±8.32	0.496
(range)	(47.30-69.00)	(55.00-75.00)	
BMI (kg/m^2) [a]	22.65±2.31	23.68±2.30	0.495
(range)	(19.94-24.94)	(20.20-26.72)	
CBZ dose (mg/day) [a]	1,050±191.48	857.14±377.96	0.372
(range)	(800-1,200)	(400-1,400)	
(mg/kg/day) [a]	17.52±0.51	13.29±5.27	0.078
(range)	(16.91-18.12)	(6.23-20.00)	
CBZ level (mg/L) [a]	6.60±2.84	7.39±2.35	0.631
(range)	(3.80-10.50)	(3.70-9.90)	
(mcg/L/mg) [a]	6.23±2.10	9.28±2.40	0.064
(range)	(3.80-8.75)	(6.29-12.50)	
CBZ clearance (L/hr) [a]	5.14±1.88	3.34±0.89	0.055
(range)	(3.33-7.68)	(2.33-4.64)	
(L/day) [a]	123.32±44.95	80.06±21.46	0.055
(range)	(80.00-184.21)	(56.00-111.36)	
(L/kg/hr) [a]	0.089±0.038	0.053±0.016	0.050
(range)	(0.048-0.139)	(0.035-0.078)	
(L/kg/day) [a]	2.13±0.91	1.27±0.37	0.050
(range)	(1.16-3.34)	(0.83-1.87)	

[a] independent t-test.

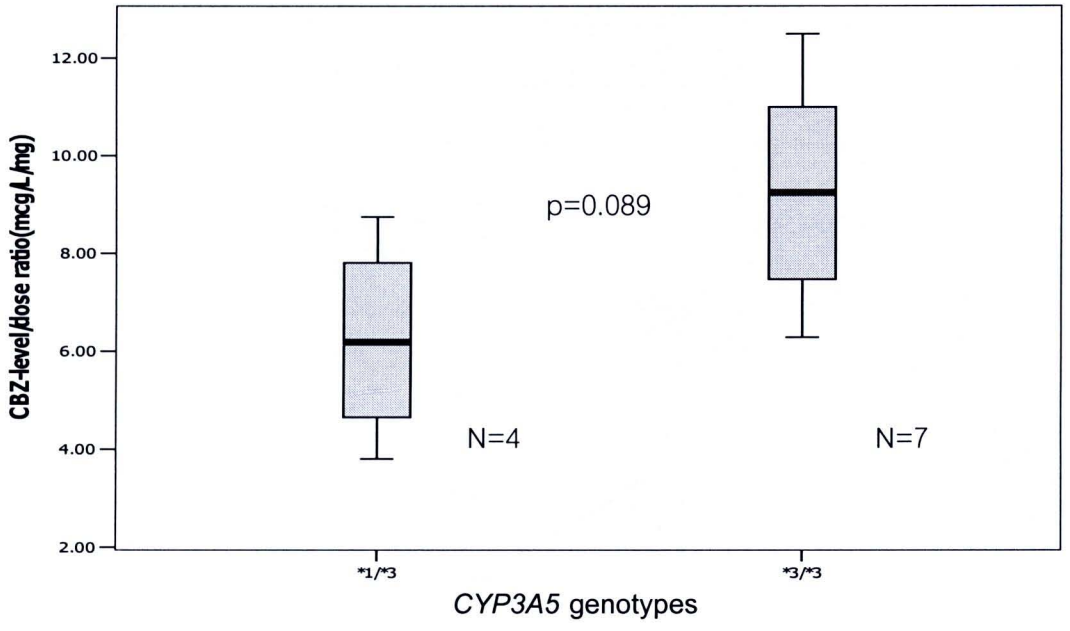


Figure 11: Box and whisker plot of the median CBZ level (mcg/L/mg) between different genotypes in CBZ+PB group (N=11).

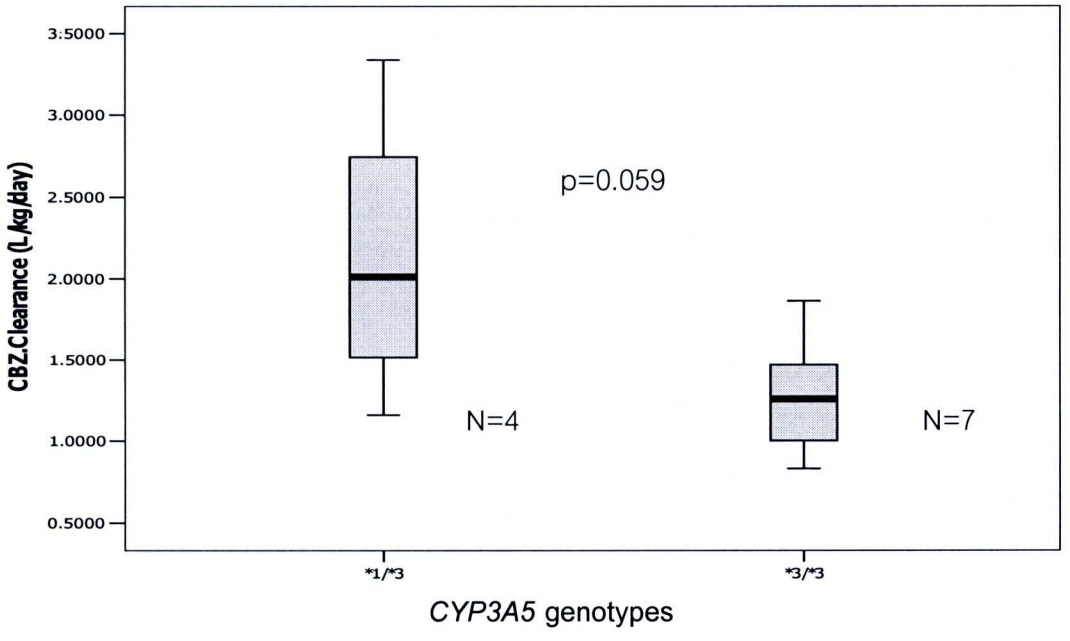


Figure 12: Box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes in CBZ+PB group (N=11).

Table 36: Comparison of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ+VPA group between *CYP3A5 *1/*1* and**1/*3* VS *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and **1/*3* (N=8)	*CYP3A5*3/*3* (N=8)	
Age (yr) [a]	32.43±11.65	41.62±16.88	0.226
(range)	(18.35-54.65)	(23.18-65.51)	
Body weight (kg) [a]	66.09±10.73	68.09±18.21	0.793
(range)	(53.00-88.00)	(43.30-104.00)	
BMI (kg/m^2) [b]	25.42	25.73	0.529
(range)	(20.02-27.06)	(16.50-37.53)	
CBZ dose (mg/day) [a]	1,000±427.62	1,100±385.45	0.631
(range)	(400-1,600)	(600-1,600)	
(mg/kg/day) [a]	15.50±7.63	16.72±6.96	0.745
(range)	(7.08-30.19)	(9.30-32.33)	
CBZ level (mg/L) [a]	8.52±2.17	7.96±0.93	0.510
(range)	(3.70-10.90)	(6.60-9.30)	
(mcg/L/mg) [a]	9.34±2.87	7.96±2.63	0.335
(range)	(5.81-13.83)	(5.36-13.17)	
CBZ clearance (L/hr) [a]	3.41±1.10	3.98±1.13	0.325
(range)	(2.11-5.02)	(2.22-5.44)	
(L/day) [a]	81.85±26.46	95.50±27.19	0.326
(range)	(50.60-120.43)	(53.16-130.67)	
(L/kg/hr) [a]	0.054±0.022	0.061±0.023	0.511
(range)	(0.027-0.095)	(0.034-0.111)	
(L/kg/day) [a]	1.28±0.52	1.46±0.54	0.511
(range)	(0.66-2.27)	(0.82-2.66)	

[a] independent t-test, [b] Mann-Whitney U Test..

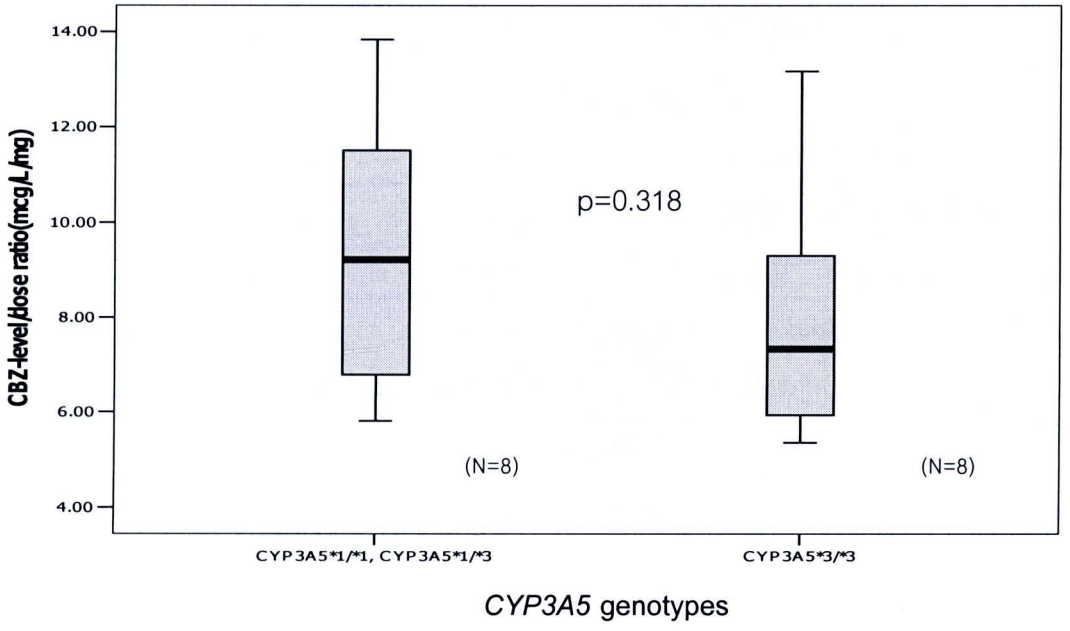


Figure 13: Box and whisker plot of the median CBZ level (mcg/L/mg) between different genotypes in CBZ+VPA group (N=16).

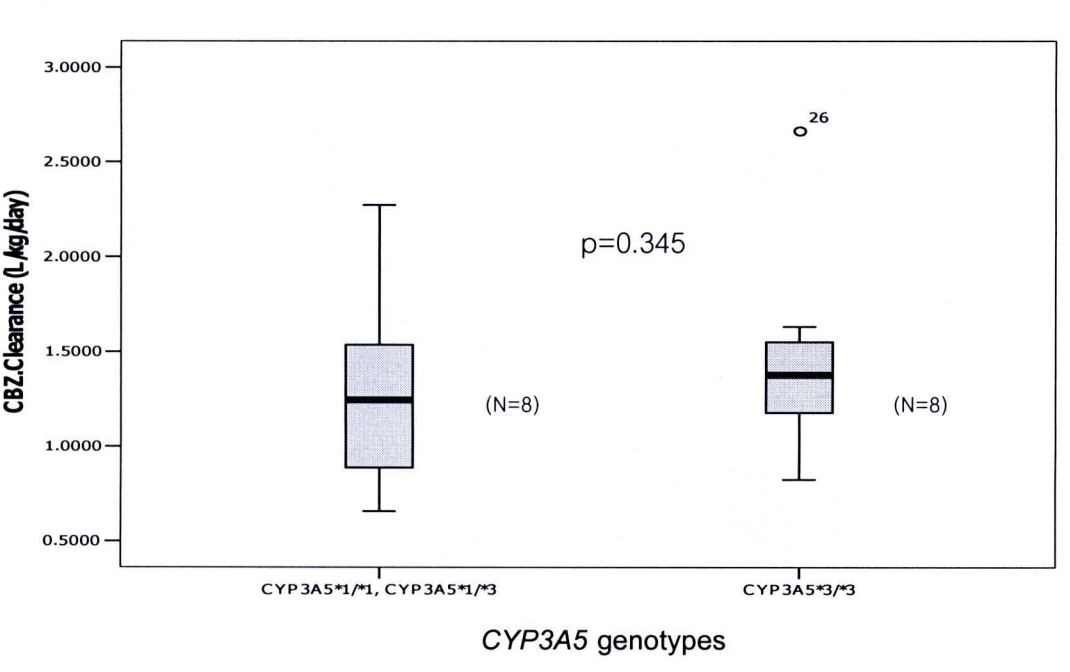


Figure 14: Box and whisker plot of the median CBZ clearance (L/kg/day) between different genotypes in CBZ+VPA group (N=16).

Table 37: Comparisons of patient's characteristics and pharmacokinetic parameters of CBZ in CBZ in combination with enzyme inducing AED group (PHT and PB) between *CYP3A5 *1/*3* and *CYP3A5*3/*3*

Parameter	Mean±SD or Median		p-value
	*CYP3A5*1/*3* (N=7)	*CYP3A5*3/*3* (N=11)	
Age (yr) [a]	46.66±10.56	45.69±8.90	0.838
(range)	(33.16-64.90)	(32.58-61.69)	
Body weight (kg) [a]	63.34±9.90	67.56±10.07	0.396
(range)	(47.30-77.00)	(55.00-82.00)	
BMI (kg/m^2) [a]	24.13±2.95	25.78±4.39	0.394
(range)	(19.94-29.34)	(20.20-34.13)	
CBZ dose (mg/day) [a]	971.43±335.23	909.09±478.44	0.768
(range)	(400-1,400)	(400-2,000)	
(mg/kg/day) [b]	17.39	11.86	0.497
(range)	(5.19-20.90)	(6.23-24.69)	
CBZ level (mg/L) [a]	5.76±2.78	6.85±2.58	0.406
(range)	(2.10-10.50)	(2.20-9.90)	
(mcg/L/mg) [a]	6.21±2.74	8.40±3.25	0.161
(mcg/L/mg) [b]	5.50	8.25	0.189
(range)	(2.63-10.50)	(3.70-13.14)	
CBZ clearance (L/hr) [a]	5.69±2.88	4.08±1.83	0.164
(range)	(2.78-11.11)	(2.22-7.88)	
(L/day) [a]	136.46±69.09	97.84±43.96	0.164
(range)	(66.67-266.67)	(53.26-189.19)	
(L/kg/hr) [a]	0.094±0.052	0.059±0.021	0.139
(range)	(0.036-0.185)	(0.035-0.097)	
(L/kg/day) [a]	2.25±1.25	1.43±0.51	0.139
(L/kg/day) [b]	1.92	1.35	0.135
(range)	(0.87-4.44)	(0.83-2.34)	

[a] independent t-test, [b] Mann-Whitney U test.

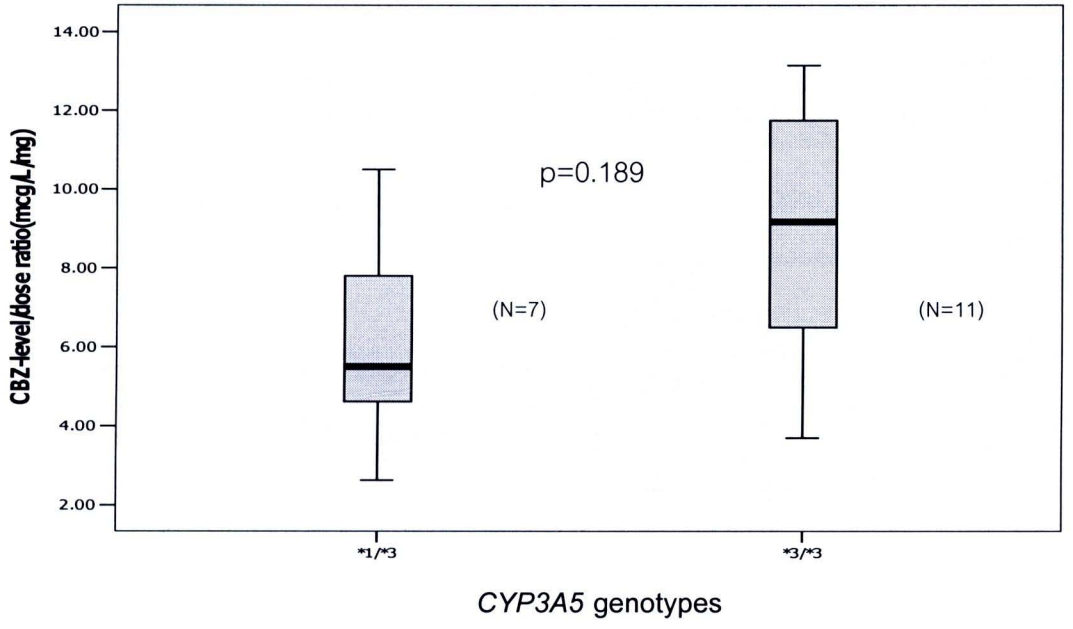


Figure 15: Box and whisker plot of median CBZ level (mcg/L/mg) between different genotypes in CBZ concurrently used with enzyme inducing AED group (N=18).

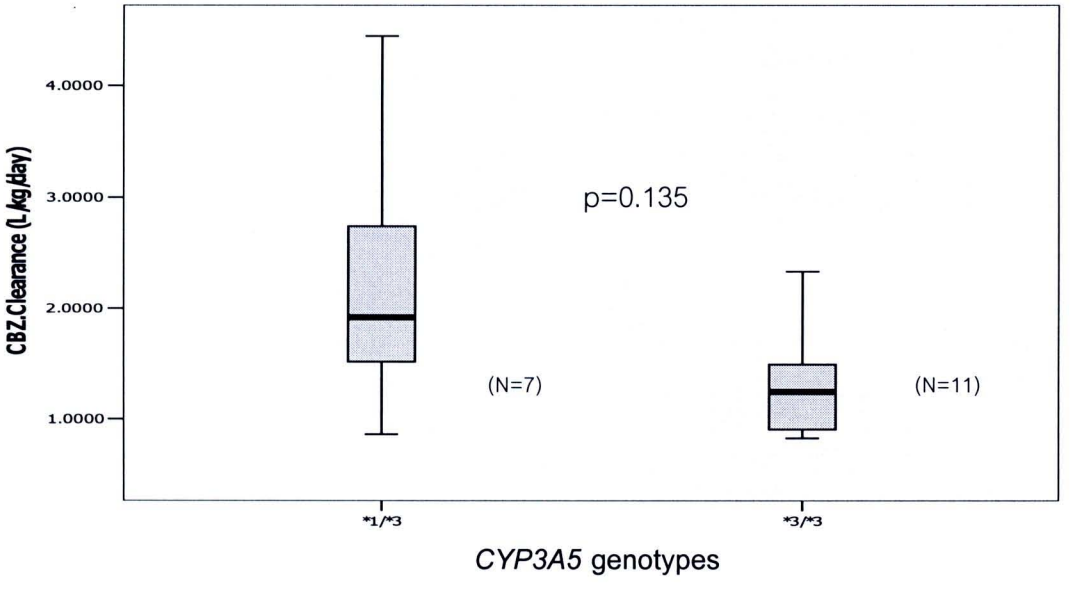


Figure 16: Box and whisker plot of median CBZ clearance (L/kg/day) between different genotypes in CBZ concurrently used with enzyme inducing AED group (N=18).

Table 38 shows comparisons of PK parameters of other AEDs used in combination with CBZ when categorized patients into 2 groups based on *CYP3A5* genotypes; the first group was *CYP3A5*1/*1* or *CYP3A5*1/*3*, and the second group was *CYP3A5*3/*3*. The PK parameters of PHT, PB and VPA (dose, level, PHT Vmax, PB clearance and VPA clearance) were not significantly different between these 2 groups of different genotypes.

Table 38: Comparisons of PK parameters of other AEDs used in combination with CBZ when categorized patients into 2 groups based on *CYP3A5* genotypes

PK parameters of other AEDs	Mean±SD or Median		p-value
	*CYP3A5*1/*1* and *CYP3A5*1/*3*	*CYP3A5*3/*3*	
CBZ+PHT (N=7)	(N=3)	(N=4)	
PHT dose (mg/day) [a]	300	325	0.150
(mg/kg/day) [b]	3.90±0.58	4.40±0.68	0.352
PHT level (mg/L) [b]	10.47±7.92	15.14±8.31	0.487
(mg/L/mg) [b]	0.0379±0.0239	0.0462±0.0209	0.646
PHT Vmax (mg/day) [b]	367.13±73.20	403.13±84.62	0.583
(mg/kg/day) [b]	5.45±1.22	5.43±0.66	0.975
CBZ+PB (N=11)	(N=4)	(N=7)	
PB dose (mg/day) [b]	120.00±48.99	83.57±45.71	0.246
(mg/kg/day) [b]	2.06±0.82	1.32±0.78	0.173
PB level (mg/L) [b]	22.75±7.24	16.50±9.13	0.273
(mg/L/mg) [b]	0.22±0.13	0.20±0.02	0.788
PB clearance (L/day) [b]	5.17±2.67	4.55±0.52	0.677
(L/kg/day) [b]	0.0935±0.0633	0.0719±0.0091	0.545
CBZ+VPA (N=16)	(N=8)	(N=8)	
VPA dose (mg/day) [b]	1,137.50±370.09	1,331.25±319.53	0.281
(mg/kg/day) [b]	17.61±6.93	20.89±8.50	0.412
VPA level (mg/L) [b]	56.70±24.57	68.41±16.00	0.278
(mg/L/mg) [b]	0.0520±0.0257	0.0545±0.0193	0.828
VPA clearance (L/day) [b]	24.12±11.76	20.89±8.75	0.543
(L/kg/day) [a]	0.36	0.27	0.834

[a] Mann-Whitney U test, [b] independent t-test.

Table 39, 40 show the comparisons of PK parameters of CBZ in the same genotype groups (*CYP3A5*1/*1* or *CYP3A5*1/*3* and *CYP3A5*3/*3*) when categorized patients into 4 groups based on other AEDs used in combination with CBZ; CBZ monotherapy, CBZ+PHT, CBZ+PB and CBZ+VPA.

Among the *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes group, CBZ dose (mg/day, mg/kg/day), CBZ level (mg/L), and CBZ clearance (L/kg/hr, L/kg/day) were not significantly different among the 4 groups categorized based on other AEDs used in combination with CBZ, while the median of CBZ level-to-dose ratio (mcg/L/mg) and the median of CBZ clearance (L/hr, L/day) were significantly different (p=0.018) between CBZ monotherapy group and CBZ+PB group (10.75 mcg/L/mg, 2.71 L/hr and 65.12 L/day VS 6.19 mcg/L/mg, 4.77 L/hr and 114.54 L/day, respectively). The details were shown in Table 39.

Among the *CYP3A5*3/*3* genotype group, CBZ dose (mg/day, mg/kg/day), CBZ level (mg/L, mcg/L/mg), and CBZ clearance (L/hr, L/day, L/kg/hr, L/kg/day) were not significantly different among the 4 groups categorized based on other AEDs used in combination with CBZ. The details were shown in Table 40.

Table 39: Comparisons of pharmacokinetic parameters of CBZ among CBZ monotherapy group and difference combination therapy groups (*CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes)

Parameter	Mean±SD or median				P- value
	CBZ (N=21)	CBZ+PHT (N=3)	CBZ+PB (N=4)	CBZ+VPA (N=8)	
CBZ dose (mg/day) [a]	800	800	1,100	1,000	0.169
(mg/kg/day) [b]	13.98±5.72	13.14±7.86	17.52±0.51	15.50±7.63	0.687
CBZ level (mg/L) [a]	8.60	4.20	6.05	9.25	0.158
(mcg/L/mg) [a]	10.75 [C]	5.44	6.19 [C]	9.22	0.030*
CBZ clearance (L/hr) [a]	2.71 [C]	5.36	4.77 [C]	3.16	0.030*
(L/day) [a]	65.12 [C]	128.61	114.54 [C]	75.88	0.028*
(L/kg/hr) [a]	0.048	0.080	0.084	0.052	0.153
(L/kg/day) [a]	1.16	1.92	2.01	1.25	0.153

* Statistical significant difference, [a] Kruskal- Wallis H test, [b] One-way ANOVA, [C] Mann-Whitney U test between CBZ VS CBZ+PB group; p-value = 0.018.

Table 40: Comparisons of pharmacokinetic parameters of CBZ among CBZ monotherapy group and difference combination therapy groups (*CYP3A5*3/*3* genotype)

Parameter	Mean±SD or median				P- value
	CBZ (N=15)	CBZ+PHT (N=4)	CBZ+PB (N=7)	CBZ+VPA (N=8)	
CBZ dose (mg/day) [a]	866.67±335.23	1,000±678.23	857.14±377.96	1,100±385.45	0.550
(mg/kg/day) [b]	14.49	10.81	14.54	14.72	0.786
CBZ level (mg/L) [a]	8.39±2.51	5.92±3.05	7.39±2.35	7.96±0.93	0.281
(mcg/L/mg) [b]	9.92	5.26	9.25	7.34	0.107
CBZ clearance (L/hr) [b]	2.94	5.70	3.15	4.04	0.108
(L/day) [b]	70.59	136.69	75.68	96.87	0.109
(L/kg/hr) [a]	0.049±0.013	0.071±0.028	0.053±0.016	0.061±0.023	0.168
(L/kg/day) [a]	1.18±0.32	1.70±0.67	1.27±0.37	1.46±0.54	0.170

[a] One-way ANOVA, [b] Kruskal- Wallis H test.

Model for prediction of carbamazepine clearance and level-to-dose ratio

Multiple regression analysis with forward-inclusion method was performed to create the model for prediction of CBZ clearance and level-to-dose ratio (mcg/L/mg) from demographic data and *CYP3A5* genotypes. Among the 70 patients participated in this study, there were only 4 factors related to CBZ clearance (L/hr and L/day) including CBZ dose (mg/kg), PHT dose (mg/kg), PB dose (mg/kg) and body weight (kg). It was found that when generated the equation to predict CBZ clearance from the related factors, the error (observed value – predicted value) was not normal distribution, when the CBZ clearance was transformed using log transformation (ln CBZ clearance), then, the error was normally distributed. Table 41A shows the entire significant models for prediction of CBZ clearance from forward-inclusion linear regression, the model 4 was the best fit equation.

There were only 4 factors related to CBZ clearance (L/kg/day) including CBZ dose (mg/kg), PHT dose (mg/kg), PB dose (mg/kg) and body weight (kg). It was found that when generated the equation to predict CBZ clearance from the related factors, the error (observed value – predicted value) was not normal distribution, then the CBZ clearance was transformed using log transformation (ln CBZ clearance) and the error was normally distributed. Table 41B shows the entire significant models for prediction of CBZ clearance from forward-inclusion linear regression, the model 4 was the best fit equation.

Table 41A: Model summary of forward stepwise linear regression for prediction of ln CBZ Clearance (L/hr and L/day)

Model	Variable entered	R	R-square	R-square change	Sig (F change)	Model Sig (ANOVA)
1	CBZ dose (mg/kg)	0.502	0.252	0.252	<0.001	<0.001
2	CBZ dose (mg/kg) PHT dose CBZ dose	0.646	0.417	0.165	<0.001	<0.001
3	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg)	0.685	0.470	0.053	0.013	<0.001
4	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg) Body weight (kg)	0.725	0.525	0.055	0.008	<0.001

Table 41B: Model summary of forward stepwise linear regression for prediction of ln CBZ Clearance (L/kg/day)

Model	Variable entered	R	R-square	R-square change	Sig (F change)	Model Sig (ANOVA)
1	CBZ dose (mg/kg)	0.639	0.408	0.408	<0.001	<0.001
2	CBZ dose (mg/kg) PHT dose CBZ dose	0.674	0.455	0.046	0.020	<0.001
3	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg)	0.714	0.510	0.056	0.008	<0.001
4	CBZ dose (mg/kg) PHT dose (mg/kg) PB dose (mg/kg) Body weight (kg)	0.740	0.547	0.037	0.024	<0.001

The coefficients and p-value of each variables which entered by forward-inclusion method of model 4 to predict CBZ clearance (L/hr and L/day) were presented in Table 42A. Multicolinearity of independent factors was determined (data not shown).

The coefficients and p-value of each variables which entered by forward-inclusion method of model 4 to predict CBZ clearance (L/kg/day) were presented in Table 42B. Multicolinearity of independent factors was determined (data not shown).

Table 42A: Coefficients of factors in the best fit equation for prediction of ln CBZ Clearance (L/hr and L/day)

Factor	B	Sig (p-value)	95% CI
For predict ln CBZ CL (L/hr)			
Constant	0.01	0.964	(-0.436)-(0.457)
CBZ dose (mg/kg)	0.04	<0.001	0.028-0.051
PHT dose (mg/kg)	0.117	<0.001	0.062-0.171
PB dose (mg/kg)	0.142	0.007	0.04-0.244
Body weight (kg)	0.008	0.008	0.002-0.014
For predict lnCBZ CL (L/day)			
Constant	3.188	< 0.001	2.741-3.635
CBZ dose (mg/kg)	0.04	< 0.001	0.028-0.051
PHT dose (mg/kg)	0.117	<0.001	0.062-0.172
PB dose (mg/kg)	0.142	0.007	0.040-0.244
Body weight (kg)	0.008	0.008	0.002-0.014

Table 42B: Coefficients of factors in the best fit equation for prediction of ln CBZ Clearance (L/kg/day)

Factor	B	Sig (p-value)	95% CI
Constant	-0.018	0.934	(-0.458)-(0.421)
CBZ dose (mg/kg)	0.042	< 0.001	0.031-0.054
PHT dose (mg/kg)	0.091	0.001	0.038-0.145
PB dose (mg/kg)	0.138	0.008	0.038-0.239
Body weight (kg)	-0.007	0.024	(-0.013)-(-0.001)

The estimation equations of CBZ clearance were shown below:

ln CBZ clearance (L/hr) = (0.04) [CBZ dose (mg/kg)] + (0.117) [PHT dose (mg/kg)] + (0.142) [PB dose (mg/kg)] + (0.008)(BW) + 0.01

ln CBZ clearance (L/day) = (0.04) [CBZ dose (mg/kg)] + (0.117) [PHT dose (mg/kg)] + (0.142) [PB dose (mg/kg)] + (0.008)(BW) + 3.188

ln CBZ clearance (L/kg/day) = (0.042) [CBZ dose (mg/kg)] + (0.091) [PHT dose (mg/kg)] + (0.138) [PB dose (mg/kg)] - (0.007)(BW) - 0.018

As shown in Figure 17, the correlation between observed ln CBZ clearance and predicted ln CBZ clearance (L/hr) was moderately significant (R-square=52.5%, $p<0.001$).

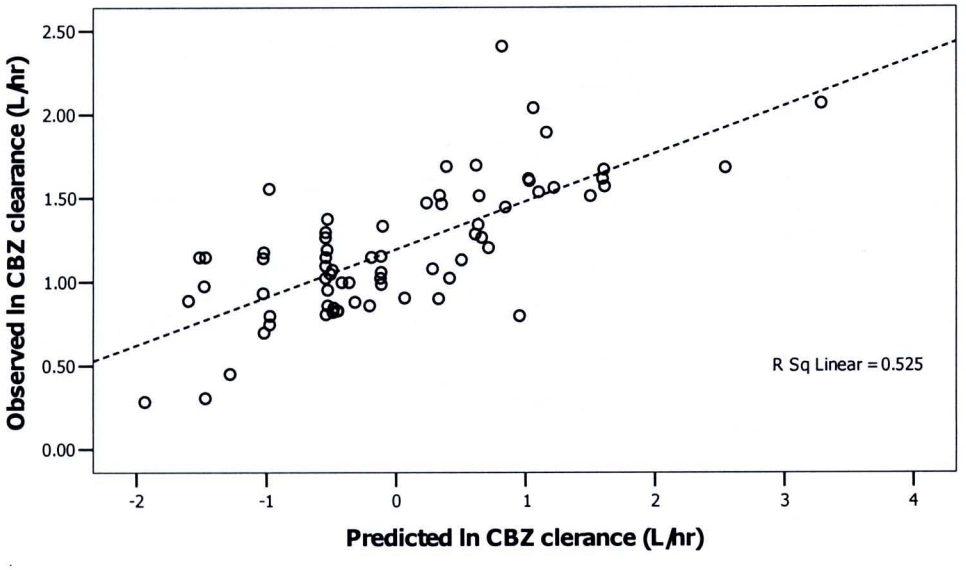


Figure 17: Scatter plot of observed ln CBZ clearance and predicted ln CBZ clearance (L/hr)

There were only 4 factors related to CBZ level-to-dose ratio including CBZ dose (mg/kg), body weight (kg), PHT dose (mg/kg) and PB dose (mg/kg).

Table 43 shows the entire significant model for prediction of CBZ level-to-dose ratio from forward stepwise linear regression, the model 4 was the best fit equation.

Table 43: Model summary of forward stepwise linear regression for prediction of CBZ level-to-dose ratio (mcg/L/mg)

Model	Variable entered	R	R-square	R-square change	Sig (F change)	Model Sig (ANOVA)
1	CBZ dose (mg/kg)	0.527	0.277	0.277	<0.001	<0.001
2	CBZ dose (mg/kg) Body weight (kg)	0.614	0.377	0.100	0.002	<0.001
3	CBZ dose (mg/kg) Body weight (kg) PHT dose (mg/kg)	0.661	0.436	0.059	0.011	<0.001
4	CBZ dose (mg/kg) Body weight (kg) PHT dose (mg/kg) PB dose (mg/kg)	0.698	0.487	0.051	0.014	<0.001

The coefficients and p-value of each variables which entered by forward stepwise method of model 4 were presented in Table 44. Multicolinearity of independent factors was determined (data not shown).

Table 44: Coefficients of factors in the best fit equation for prediction of CBZ level-to-dose ratio (mcg/L/mg)

Factor	B	Sig (p-value)	95% CI
Constant	20.964	< 0.001	16.643-25.286
CBZ dose (mg/kg)	-0.382	< 0.001	(-0.495)-(-0.269)
Body weight (kg)	-0.084	0.006	(-0.142)-(-0.025)
PHT dose (mg/kg)	-0.8	0.004	(-1.33)-(-0.27)
PB dose (mg/kg)	-1.254	0.014	(-2.243)-(-0.265)

The estimation equation of CBZ level-to-dose ratio was show below:

CBZ level-to-dose ratio (mcg/L/mg) = (-0.382) [CBZ dose (mg/kg)] - (0.084) [BW (kg)]

- (0.8) [PHT dose (mg/kg)] - (1.254) [PB dose (mg/kg)] + 20.964

As shown in Figure 18, the correlation between observed CBZ level-to-dose ratio and predicted CBZ level-to-dose ratio was moderately significant (R-square=48.7%, $p<0.001$).

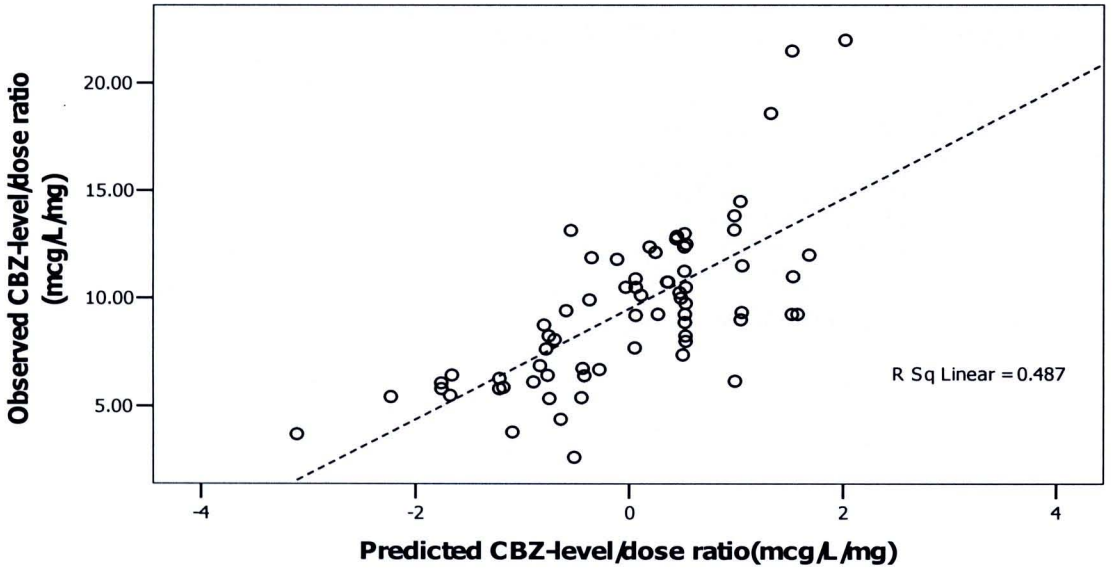


Figure 18: Scatter plot of observed CBZ level-to-dose ratio and predicted CBZ level-to-dose ratio

Interethnic variability of CYP3A5 polymorphism in Asia

Allelic frequencies of *CYP3A5* polymorphism in Asian population were different from Caucasian and African-American population. [17, 34-38] Among Asian population the allelic frequencies of *CYP3A5* polymorphism were not different (Table 45).

Table 45: Comparison of *CYP3A5* allele frequencies among Asians

Ethnicity	Number of subject	% Allele frequency		p-value (compared to this study)
		*1	*3	
Thai (This study)	**70**	**31**	**69**	-
Thai [17]	150	34	66	0.65
Chinese [34]	302	22	78	0.15
Indian [35]	90	41	59	0.14
Malaysian [35]	98	39	61	0.24
Japanese [36]	200	23	77	0.21