Influence of ethnicity in optimizing antiepileptic drug dosing: a comparison of Malay, Chinese and Indian populations in Malaysia

by

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Abstract

Reports of inter-ethnic differences in metabolism for phenytoin and carbamazepine have raised questions concerning the importance of monitoring serum levels to the standardised population therapeutic concentrations. Although the pharmacokinetics of phenytoin, carbamazepine, valproic acid and phenobarbitone displayed both intra and inter-individual variations, the influence of ethnicity is still unclear. This thesis has thus set its objectives of investigating the impact of ethnicity on the efficacy of these therapeutic ranges and pharmacokinetics of these drugs.

A total 1554 serum concentrations were randomly selected by a set of criteria from 470 Malays, 423 Chinese and 322 Indian of adult and paediatric patients. The Mantel-Haenzel method was used to estimate for inter-ethnic differences in response to the defined therapeutic ranges. The influence of ethnicity on pharmacokinetics was examined by the test of heterogeneity of the slopes estimates in the linear relationship of either serum concentration or clearance to dose. Coefficient of variation on the ratios of the above relationships was used to measure for inter-individual variation.

The results showed a highly variable response to treatment within the defined therapeutic ranges. Therapeutic response is not dependent on ethnicity and age although the latter was determined on carbamazepine and valproic acid treated patients only. The pharmacokinetics of carbamazepine, valproic acid and phenobarbitone showed high inter-individual variations and were unaffected by weight, age or ethnicity. Similar high inter-individual variation for phenytoin pharmacokinetic parameters (*Km* and *Vmax*) were observed. However, *Km* and *Vmax(mg/day)* of adult Chinese patients were significantly lower than Malay or Indian patients. The relationship between *Km* and *Vmax* and age or weight were insignificant. These findings demonstrate that Malaysian patients only differed in handling phenytoin therapy and support the use of ethnic specific phenytoin pharmacokinetic parameters during therapy.

Research summary

The measurement of drug concentrations of established antiepileptic drugs such as phenytoin, carbamazepine, valproic acid and phenobarbitone in clinical practice is essentially to monitor patient response within a predetermined range or target range. Although these target ranges have been well documented, the influence of ethnic differences on the degree of effectiveness of these drugs have yet to be fully explored. Review of the literatures gave evidences that inter-ethnic differences in metabolism are confirmed for phenytoin, possible for carbamazepine and less likely in valproic acid and phenobarbitone.

Pharmacokinetically, the relationship between dose and serum concentration of carbamazepine, valproic acid and phenobarbitone has still to be confirmed and high intra and inter-individual variation have being quoted as reasons for inconsistencies between studies. The relationship between inter-individual variation and inter-ethnic differences among ethnic groups is still uncertain. This thesis thus undertakes to examine these uncertainties by investigating the impact of inter-ethnic differences on the drugs present therapeutic concentrations and to determine whether factors such as ethnicity, age, weight, etc, can influence the steady-state pharmacokinetics of these drugs.

Retrospective routine data of serum concentrations of patients taking either one or more of the studied drugs from the years of 1993 to 1996 were retrieved from five government run hospitals in Malaysia. Patients were selected based on a set of inclusion and exclusion criteria. A total of 1,215 from the 1,868 patients screened met the set criteria of which 874(71.93%) were adults and 341(28.07%) were children. Reasons for exclusion of about 34.96% of the shortlisted patients were inadequate patient data, non-compliance and lost files. The inter-ethnic composition comprises of 470 Malays, 423 Chinese and 322 Indians.

To evaluate the effectiveness of the documented target ranges of the four studied drugs on the control of seizures and to determine for inter-ethnic differences in response, the Mantel-Haenzel fixed effect model of odds ratios and for pooling across data was used. This method basically measures the odds-ratio of controlled to uncontrolled patients based on a random selection of a single dose and serum concentration pair and the subsequent seizure index of each drug. Seizure index is the rate of seizures per day at each hospital review. The estimated summary estimate of the pooled odds-ratio and the 95% confidence limits was used to test for heterogeneity across the three ethnic populations.

The same data set was used to investigate the steady-state pharmacokinetics of carbamazepine, valproic acid and phenobarbitone. To examine inter-ethnic differences, the formula that determines the relationship of serum concentration and dose by Wagner (1965) was employed. Test of heterogeneity between ethnic groups was by examining the differences of the pooled estimates of the slope of the significant linear regression equation of either dose and serum concentration or

clearance was used. Phenytoin pharmacokinetics were determine by the Michaelis-Menten equation which uses two dose and serum concentration data sets.

The differences in variables such as age, weight, sex, disease duration(length of disease) and epilepsy types was determined by one way analysis of variance. P value of 0.05 or less is set to test to significance. Coefficient of variation of mean values of serum/level ratio was used for interindividual variation.

Results showed that routine monitoring of established antiepileptic drugs in relation to their target ranges is clinically ineffective and had little bearing to achieving good therapeutic response. Therapeutic response, which was measured by the degree of seizure control was not dependent on inter-ethnic differences. These results were confirmed as the differences observed between Malays, Chinese and Indians pooled odds ratio of controlled and uncontrolled patients in the defined therapeutic ranges were statistically insignificant. High inter-individual variation was clearly apparent in all ethnic groups showing that the use of these drugs should be individualised. Age was not an important factor for the association between therapeutic response and the target therapeutic range. This was confirmed by the pooled odds ratio of carbamazepine and valproic acid. Similar observation were not conclusive for phenytoin and phenobarbitone due to inadequacy of data. These observations are thus in-line with the recommendation outlined by the Commission on Antiepileptic drugs, International League Against Epilepsy 1993.

The relationship between dose and serum level for carbamazepine is found to be curvilinear and thus verified its dose-dependent properties. Linear relationship between valproic acid dose and serum level is highly variable and found to be highly significant in patients on valproic acid monotherapy. Phenobarbitone dose and serum concentration relationship are highly correlated. This study also showed that neither valproic acid nor phenobarbitone displayed any dose-dependent kinetics.

Factors such as weight, age or ethnicity have no influence on the relative relationship between dose and serum concentration for carbamazepine, valproic acid and phenobarbitone. The main factor that could have affected this relationship is the high interindividual variation between patients which clearly indicates that the use of these drugs must be individualised.

Clearance is the pharmacokinetic parameter used in determining the trend and influence of ethnicity on the population pharmacokinetics of carbamazepine, valproic acid and phenobarbitone. Evidence of inter-ethnic differences among Malay, Chinese and Indian epileptic population in Malaysia was found to be statistically insignificant. High coefficient of variation of clearances displayed in all ethnic groups showed that interindividual variation is the important factor that could affect the relationship between clearance and dose.

Evidence showed that there are inter-ethnic differences in phenytoin pharmacokinetic parameters (*Km,mg/L* and *Vmax,mg/day*) among Malay, Chinese and Indian adults patients of Malaysia but not for paediatric patients. Elimination rate constant with a value equal to the plasma concentration at which the rate is one-half the maximum (*Km*) was found to be lower for Chinese and differed significantly to

that of Malay and Indian adult patients. Differences between Malay and Indian adult patients were insignificant. Metabolic rate capacity (*Vmax,mg/kg.day*) of all three ethnic groups were statistically similar.

The relationship between *Km* with age and weight in both adult and paediatric patients were non-linear. Relationship between *Vmax (mg/kg.day)* with age or weight was weak although there is an inclination towards a linear relationship in paediatric patients.

Finally, this study showed that Malay, Chinese and Indian patients did not differ significantly in handling carbamazepine, valproic acid and phenobarbitone. However, inter-ethnic differences between Chinese with both Malay and Indian patients signify that the therapeutic use of phenytoin on Malaysian patients must be adjusted to their racial background.

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Part 1

Background

CHAPTER 1

ANTIEPILEPTIC DRUGS : AN INTRODUCTION

1.1 BRIEF HISTORY

Epilepsy is a disease that has affected people for as long as history has been recorded. The term 'epilepsy' is derived from a Greek word that means to take hold of, to possess or to seize. The ancient Greeks' belief was that an epileptic attack represented possession by the gods. By the year 400 BC, Hippocratic writers identified epilepsy as a physical disorder of the brain, and pointed out that damage of one side of the brain can lead to convulsions that affect mainly the opposite side of the body. It was later defined by a British neurologist Hughlings Jackson about a century ago as a recurrent, episodic, uncontrolled discharge of nerve tissue [Chadwick and Usiskin 1987].

Epidemiologically, about 0.5 to 1% of the population in the United Kingdom have recurrent seizures at some time of their lives. Chadwick and Usiskin (1987) further describe that incidence is highest at the first ten years of life while prevalence increases with age.

In the United States of America, the prevalence of active epilepsy is 6.42 cases per 1000 population. In the 1980 census, at least 1.5 million people in America have active seizures [Scheuer and Pedley 1990]. Active seizures such as status epilepticus, which is characterised by a succession of fits without regaining consciousness between them, may cause brain damage. This is because the brain cells may become so overactive that the blood cannot transport enough oxygen to the brain and lead to these cells dying. The commonest reason as to why this happens is that patient stop taking the medication [Chadwick and Usiskin 1987]. Thus, the goal of drug therapy is to minimise the recurrence of seizures by ensuring compliance with no unwanted side-effects.

1.2 WHAT IS EPILEPSY?

Epilepsy is defined as a condition characterized by recurrent (two or more) epileptic seizures, unprovoked by any immediately known cause. It is usually a chronic disorder characterized not only by recurrent seizures but by a variety of medical and psychosocial implications. Manifestation usually consists of sudden and transitory

abnormal phenomena, which may include alterations of consciousness, motor, sensory, autonomic, or psychic events perceived by the patient or an observer. Its clinical manifestation is presumed to result from an abnormal and excessive discharge of a set of neurons in the brain.[Hopkins and Shorvon 1995]

According to the International League Against Epilepsy, 1981, epilepsy can be broadly classified into four main groups. The first group of epilepsy are the localization-related (focal, local, partial) epilepsies and related syndromes. The other three groups are the generalized epilepsies and syndromes, epilepsies and syndromes undetermined whether focal or generalized and finally epilepsies due to special syndromes. Special syndromes are epilepsies that are usually situation-related. Examples are febrile convulsions, isolated seizures and those that occur only when there is an acute metabolic or toxic event. Table 1.1 summarised the modified classification by the Commission in 1989.

Table 1-1: ***International classification of epilepsies and epileptic syndroms***

I.	Localization-related (focal, local, partial) epilepsies and epileptic syndroms	
	a.	Idiopathic with age-related onset
		1. Benign childhood epilepsy with centrotemporal spikes
		2. Childhood epilepsy with occipital paroxyms
	b.	Symptomatic
II.	Generalized epilepsies and epileptic syndroms	
	a.	Idiopathic with age-related onset
		1. Benign neonatal epilepsy
		2. Childhood absence epilepsy (Pyknolepsy)
		3. Juvenile myoclonic epilepsy (impulsive petit mal)
		4. Juvenile absence epilepsy with generalized tonic-clonic seizures on awakening.
	b.	Secondary (idiopathic or symptomatic)
		1. West syndrome (infantile spasms)
		2. Lennox-Gastaut syndrome
	c.	Symptomatic
		1. Nonspecific etiology (early myoclonic encephalopathy)
		2. Specific syndromes (epileptic seizures that may complicate many diseases, e.g Ramsay Hunt syndrome, Unverricht's disease)

Modified and abbrevaited from Commission(1989) -(Mattson RH 1995)

As for seizure types, there are categorically classified into three main groups [International League Against Epilepsy, 1989]. These are partial seizures beginning locally, generalized seizures (convulsive or nonconvulsive) and lastly seizures that are unclassified.(Table 1-2) The unclassified epileptic seizures include some neonatal seizures which can feature as rhythmic eye movements, chewing and swimming movements.

Table 1-2 : International classification of epileptic seizures

I.	Partial (focal, local) seizures	
	a.	Simple partial seizures
	b.	Complex partial seizures
		1. with impairment of consciousness at onset
		2. simple partial onset followed by impairment of consciousness
	c.	Partial seizures evolving to generalized tonic-clonic seizures (GTCS)
		1. Simple partial seizures evolving to GTCS
		2. Complex partial seizures evolving to GTCS, including those with simple partial onset
II.	Generalized seizures (convulsive or nonconvulsive)	
	a.	Absence seizures
	b.	Atypical absence seizures
	c.	Myoclonic seizures
	d.	Clonic-tonic seizures
	e.	Tonic clonic seizures
	f.	Atonic seizures
III.	Unclassified epileptic seizures, including some neonatal seizures	

Modified and adapted from commission (1981) - (Mattson RH 1995)

1.3 ANTIEPILEPTIC DRUGS

Antiepileptic drugs are drugs that are used in the treatment of epilepsy. They are usually administered over a long period, and will decrease the incidence or severity of spontaneously seizures occurring in patients with epilepsy. Among the commonly used or established antiepileptic drugs are phenobarbitone, phenytoin, carbamazepine and sodium valproate [Gardner-Thorpe et al 1977]. Phenobarbitone was the earliest drug to be used in the treatment of epilepsy in 1912 while sodium valproate was licensed for clinical use in the United Kingdom in 1973. The four drugs are structurally different and their mechanisms of actions are still not clearly defined.

The use of newer generation AED such as vigabatrin, gabapentin, lamotrigine topiramate, felbamate etc are however dependent on availability and on the licensing conditions in each country. Vigabatrin, gabapentin, lamotrigine and topiramate have been used in the United Kingdom since 1996 for partial and secondarily generalized seizures. All four drugs are currently used in adults while vigabatrin and topiramate are also recommended for use in children [BNF 1996]. As for the United States, these four drugs and felbamate have being approved for use since 1996 [Dichter and Brodie 1996]. Others that include clobazam, oxacarbazepine, tiagabine and zonisamide are currently being approved for use in certain countries in Europe, Canada and Japan. The use of these drugs

in epilepsy are standardised worldwide and table 1-3 and 1-4 summarizes the selection of standard antiepileptic drugs and the dosage guidelines in adults and children.

1.4 MECHANISM OF ACTIONS OF ANTIEPILEPTIC DRUGS

1.4.1 The Established Few.

Carbamazepine is a iminodibenzyl derivative and chemically related to the tricyclic antidepressants. It is a neutral compound and poorly soluble in water. Phenytoin is an hydantoin derivative, poorly soluble and a weak acid. As for their mechanisms of action, both have been shown to interact with voltage-dependent sodium channels at concentrations found free in plasma [MacDonald RL 1989]. These drugs were demonstrated to reduce the frequency of sustained repetitive firing of action potential in neurons in cell culture [McLean and McDonald 1983]. Antiepileptic drug action appeared to be caused by a shift of sodium channels to an inactive state that was similar to the normally occurring inactive state but from which recovery was delayed.

Phenobarbitone is found to enhance GABA receptor (GABAR) activity by binding to an allosteric regulatory site on the receptor site on the receptor. Results from fluctuation analysis suggest that phenobarbitone increase the mean channel-open duration of GABAR currents without altering channel conductance [Olsen RW 1987, MacDonald et al 1989b, Twyman et al 1989]. Sodium valproate is a short chain fatty acid and is only slightly soluble in water. Its effect on sodium channels has been less studied but is found to block sustained high-frequency firing of neurons in culture [McLean et al 1986a].

Both phenytoin and carbamazepine have a wide spectrum of activity covering partial, generalized and unilateral seizures. Sodium valproate, although having some activity against all three types of seizures, is more well known for its efficacy against primary generalized seizures with particular value in idiopathic generalised seizures. Phenobarbitone activity is more restricted to partial seizures and tonic, clonic or tonic-clonic seizures [Shorvon S 1995].

1.4.2 The Newer Generation Antiepileptic Drugs

The newer AED such as gabapentin, lamotrigine and felbamate are used as an add-on drugs in adults with partial or secondarily generalized seizures. All the three drugs have no published target range for serum concentrations but, a minimum of 2 ug/ml and a

Table 1-3: Selection of standard entiepileptic drugs

Drug	Advantages	Disadvantages	Comment
Carbamazepine	Very effective for partial and tonic-clonic seizures;minimal sedative, cognitive, or behavioral adverse effects	Transient adverse effects during initiation of therapy; no parenteral formulation; may worsen absence seizures	A drug of first choice for partial epilepsies
Phenobarbital	Broad spectrum of efficacy; predictable absorption from parenteral or other routes of administration; inexpensive	Adverse sedative, cognitive, or behavioural effects; connective tissue effects; potency or libido problems	No longer a drug of first choice, but effective, relatively safe and inexpensive
Phenytoin	Very effective for partial and tonic-clonic seizures; parenteral formulation	Cosmetic or dysmorphic side-effects; saturation kinetics	A drug of first choice for partial epilepsies; potent enzyme inducer
Valproate	Broad spectrum of efficacy	Rare hepatic dysfunction; weight gain; tremor; teratogenicity	The drug of first choice for idiopathic epilepsy; an alternative drug of choice for partial seizures
Gabapentin	Effective in partial and tonic-clonic seizures; well tolerated	Limited absorption; short half-life; moderate efficacy	Mechanism of action not established
Felbamate	Broad spectrum of efficacy: effective in Lennox Gastaut syndrome;alerting	Rare fatal aplastic anemia and hepatitis, headache, insomia, vomiting, and weight loss	Choice limited due to risks;interacts with and inhibits metabolism of other AED
Lamotrigine	Broad spectrum of efficacy; sense of well-being	Hypersensitivity reactions; metabolism inducible	Extensive experience
Tiagabine	Effective in partial and tonic-clonic seizures	Hepatic metabolism;short half-life	Unique mechanism of action; blocks GABA reuptake
Vigabatrin	Effective in partial and tonic-clonic seizures; infantile spasms	Uncommon but apparently psychiatric symptoms	Unique mechanism of action; irreversibly inhibits GABA transaminase

Adapted from Mattson 1995

Table 1-4 : Dosage guidelines and side-effects for antiepileptic drugs in adult and children

Drug	Adult			Children			Side-effects
	Starting dose (mg/day)	Maintenance dose-(mg/day)	Dosage schedule	Starting dose (mg/kg/day)	Maintenance dose (mg/kg/day)	Dosage schedule	
Carbamazepine	100-200	400-2000	bid-qid	5	10-25	bid-tid	Drowsiness, blurred vision, diplopia, dysequilibrium, leukopenia, hepatic failure
Phenobarbital	30-60	60-240	od-bid	4	4-10	od-bid	Sedation,depression,loss of concentration, mental dulling, hyperactivity
Phenytoin	200-300	100-700	od-bid	5	5-15	od-bid	Ataxia, dysthria, gingival hypertrophy, hirsutism, acneiform eruption, hepatic failure, osteomalacia
Sodium valproate	500	500-3000	od-bid	10	15-40	od-bid	Gastrointestinal upset, mood changes, lethargy, hiccups, headache

Abbreviations: od-once daily, bid-twice daily, tid-thrice daily, qid- four times daily (Scheurer and Pedley 1990)

Table 1-5: Specific mechanisms of antiepileptic drug (AED) action

AED	Na^+	Ca^{2+}(T)	Ca^{2+} (L,N,P,O)	GABA[a]	Glutamate	Carbonic Anhydrase
Phenytoin	+++		+	+		
Carbamazepine	+++			+		
Phenobarbital	++		+	++	++	
Valproate	++	+			+	
Lamotrigine	+++		+			
Vigabatrin				+++		
Tiagabine				+++		
Gabapentin	+			++		
Felbamate	+	+		+	++	
Topiramate	++			++	++	+

+++, well-documented action believed to account for a major oart of drug's anticonvulsant effect; ++, effect probably of clinical significance; +, effect only tentatively characterized or seen at supratherapeutic concentrations.
GABA, g-aminobutyric acid,[a]GABA covers a variety of mechanism.
L,N,P,O- types of calcium channels
(adapted from Meldrum 1996)

maximum of 20ug/ml is observed to be effective for gabapentin. As for lamotrigine, serum concentrations higher than 10 ug/ml are tolerated by most patients [Dichter et al 1995].

The mechanism of action of gabapentin is still unknown, but it is observed to bind to a specific receptor in the brain, [Hill et al 1993]. This inhibits the voltage-independent sodium currents [Wamil et al 1994] and may enhance the release or actions of alpha-aminobutyric acid [Kocsis et al 1994]. Lamotrigine however has similar antiseizure and mechanism of action to that of phenytoin and carbamazepine [Cheung et al 1992]. As for felbamate, which is also prescribed in children with partial or generalized seizures associated with the Lennox-Gastaut syndrome, is thought to enhance the inhibitory actions of alpha-aminobutyric acid, and blocks N-methyl-D-aspartate receptors [Rho et al 1994]. Table 1-5 lists the mechanism of action of the antiepileptic drugs described above.

1.5 Pharmacokinetic model

By the word 'pharmacokinetic' is meant the application of kinetics to pharmacon which is a Greek word for drugs and poisons. World Health Organisation however defined pharmacokinetics as 'the study of the absorption, distribution, metabolism, and the excretion of drugs'. Objectively, the purpose of pharmacokinetics studies are to reduce data to meaningful figures or parameters, to use the reduced data to make predictors of results of future experiments or predictors of results of a host of studies.

By modelling in pharmacokinetics analysis is meant displaying results in different representations. Shahin et al 1984 defined the essence of model building as;

> *"Model building is as much an art as it is a science. It involves intuition, imagination, and skill. It is impossible to state a set of rules to build a mathematical model as much as it is not possible to draw a picture or paint a landscape following a list of regulations. It depends upon the viewpoint and judgement of the modeler to decide what information should be included or emphasized and to what extend in the model. However, it is possible to offer a set of guidelines or a framework around which the modeler can develop and improve his skill and imagination to build a model"*

Basically, there are three important reasons for modelling; ***descriptive, predictive*** and ***explanatory***. Descriptive modelling approach is when the data is described in a simple, efficient and easily communicated means. This is usually in the form of graphs

such the scatter plot or line graph where certain specific measurements such as clearance can be calculated using the area under the curve (AUC) methodology. Descriptive models are thus attempting to relate the interaction of a drug to a biological environment by choosing a mathematical model and using the data to define values of unknown model parameters. The model parameters, determined from the fit of the model to the observed data, also function as measures for comparisons of results both within and between experiments.

The second reason for modelling is its predictive capabilities. Studies on time course after multiple dosing based on a single data or determination of absorption profile of a certain drug would certainly benefited by pharmacokinetics modelling. Modelling in this case offers an easy and flexible method of predicting certain unknown relationship between two determinants.

Pharmacokinetic modelling is useful in explaining unclear or unusual observation. A clear example is the model to elucidate the outcome of complex mechanism such as the glucose-insulin model [Ackerman et al 1964 & 1965]. Other good examples are phenytoin capacity-limited metabolism [Arnold & Gerber 1969, Lund et al 1974, Chang & Glazko 1982] and carbamazepine autoinduction properties [Rapeport et al 1983, Bertilsson & Tomson 1986].

In most pharmacokinetics studies, the estimation of parameters from condensed data is critical. The quantification of parameters from the system kinetics is best assumed by some form of pharmacokinetics modelling. This is an important aspect since the parameters are actually numbers that quantitate the pharmacokinetics of the system studied.

1.5.1 Constants, parameters and variables

These expressions are commonly stated in pharmacokinetics modelling. The meaning of each can be easily explain by the following simple model;

$$C_t = \frac{D.\, e^{-kt}}{V} \qquad \textit{(eqn. 1.0)}$$

where D(dose) is a time constant of known value and stays **constant.** Volume of distribution(V) and the elimination rate constant(k) are **parameters** to be estimated by fitting the model to the data. Time(t) and model predicted concentration(C_t) are variables.

The relationship between observed and predicted variables and the measurement error(ε) is given by ;

$$C = C_d + \varepsilon \qquad (eqn.\ 1.1)$$

where C_d can be a time value or a mean value.

Since pharmacokinetics modelling involves some form of regression, the main goal is to understand how a response variable example concentration or effect, depends on one or more predictors such as dose, clearance, time, age, weight and even concentration. The response variable is called dependent variable, and the predictor variable may be called independent explanatory variable or covariate.

1.5.2 Parameter estimates

i. Linear and non-linear models

Assuming a series of concentration(Y_{ith}) and N as the number of observation being denoted by the following model;

$$Y_{ith} = f(X_d.\beta_d) + \varepsilon \qquad (eqn.1.3)$$

$$X_d = (x_1, x_2, ...) \qquad (eqn.1.4)$$

$$\beta_d = (\beta_1, \beta_2, ...) \qquad (eqn.1.5)$$

where X_d is the vector of independent variables corresponding to the observation and β_d signifies the vector of model parameters which must be estimated. The function $f(X_d.\beta_d)$ represents the true deterministic concentration corresponding to X_d and β_d, and ε denotes a random unobservable error. The distribution of error(ε) is assumed as normal, and has a mean of zero and a variance of σ^2.

$$\varepsilon = N(0, \sigma^2) \qquad (eqn.1.6)$$

Except for the magnitude of the random error ε, given the values of X and β, the exact value of Y can be determined. This model is then said to be deterministic and termed as linear if,

$$f(X_d,\beta_d) = X_d.\beta_d \qquad (eqn.1.7)$$

i.e, f is the product of X_d and β_d. Examples of linear models are;

$$Y = \beta + \beta_1.X_d \qquad (eqn.1.8)$$

$$Y = \beta + \beta_1.X_d + \beta_2.X_d^2 \qquad (eqn.1.9)$$

The first model is a simple linear model and would appear as a straight line with intercept β_0 and slope β_1. Equation 1.8 is a quadratic regression model and may not

appear as a straight line. However, this model is still considered linear because it is a linear function of the parameters of β_1 and β_2.

It is possible to determine if a model is linear by looking at the associated partial derivatives. As an example, in equation 1.8 the partial derivative of β_0 is 1, β_1 is X_d and β_2 is X_d^2. Since none of these derivatives involved β, β_1 , and β_2 , the model is said to be linear.

A non-linear model is the opposite of a linear model where the partial derivatives are inter-dependent, i.e

$$Y = \beta_0 + \beta_1 . e^{-\beta_2 X_d} \qquad \textit{(eqn.1.10)}$$

For the above equation, the partial derivative of β_1 clearly depends on β_2. Another characteristic of a non-linear model is Y cannot be expressed as a product of X_d and β_d.

Finally, linear pharmacokinetics models such as the first order kinetic models are generally non-linear regression models;

$$C(t) = D.e^{-kt} / V \qquad \textit{(eqn.1.11)}$$

in which case, k and V vis-a vis are the dependent variable of $C(t)$,while $C(t)$ and k is not directly proportional to each other. However, the non-linearity can be linearized by taking the logarithm of both sides of the equation.

1.5.3 Criteria for best fit - Minimisation methods

The three most used criteria for best fit are the **ordinary least square(OLS), weighted least squares(WLS)**, and the **extended least squares(ELS)**. These criteria are achieved by minimizing the following quantities which are often called **objective function(*O*)** ;

$$O_{OLS} = \sum_{i=1}^{n} (Ci - C_d i)^2 \qquad \textit{(eqn1.12)}$$

$$O_{WLS} = \sum_{i=1}^{n} Wi. (Ci - C_d i)^2 \qquad \textit{(eqn.1.13)}$$

$$O_{ELS} = \sum_{i=1}^{n} [Wi.\ (Ci - C_d i)^2 + lnVar(C_d i)] \quad (eqn.1.14)$$

where $C_d i$ denotes the predicted value of Ci based on the model.

The correct criteria for best fit depends upon the assumption underlying the functional form of the variances of the dependent variable C. The most commonly employed variance model is ;

$$Var(C) = \sigma^2 . f(X_d, \beta_d)^{\lambda} \quad (eqn.1.15)$$

Draper and Smith (1981) defined the theoretically correct weight based on the maximum likelihood consideration as the reciprocal of the variance of the observation. This gives large variances less weight as compared to small variances.

The value and accuracy of the three equations depend on the λ. If $\lambda = 0$, variances of C_i are identical and constant, OLS, WLS and ELS are equivalent. However, if the weights are constant (which means it does not involve any parameters which must be estimated), then estimation of parameters using WLS and ELS is equivalent.

The suitability for any type of least square methodology in modelling also depends on the function of X_d and β_d. WLS is appropriate when the variance is some function of the X_d only. If it also depends on β_d, then the modified version of the WLS least square namely called the iterative reweighted least square(IRLS) is more appropriate [Gabrielsson and Weiner 1997].

ELS, which employs a maximum likelihood procedure [Sheiner and Beal 1985], estimated the value of λ. Gabrielsson and Weiner (1997) commented that this method offers no practical advantage over IRLS and can produce parameters more biased or have greater variability than those produced by IRLS [Metzler 1987, van Houweligen 1988].

1.5.4 Pharmacokinetics compartmentalisation

The metabolism of drugs occurs mainly through the liver and is dependent on the capacity of the drug metabolism system. Drug metabolism in the liver enzymes is ***capacity limited*** and hence the kinetics are non-linear when the entire concentration range of substrate(drug) is considered. The limitation may arise because of the limited amount of

enzymes, limited amount of co-factors involved in biotransformation, and a limited amount of reactant as in the availability of sulphate in sulphate conjugation.

Two pharmacokinetics models that best describe this behavior are the ***linear compartmental*** and ***non-linear compartmental*** model. ***Linearity*** is a term defined by the indicator that for a given drug and individual all concentration-time profiles that are normalized for time and size should be superimposible. ***Non-linearity*** thus implies that such profiles are not superimposible due to one or more time and dose-related dependencies [Rowland and Tozer 1989, Tozer et al 1981]. Basically, linearity is a direct proportion of transfer rates to concentration or concentration differences. An important consequence of a linear system on pharmacokinetics is that the total area blood(plasma or serum) concentration, and time curve following intravenous administration is a linear function of the dose administered.

The compartmentalized system in pharmacokinetic modelling is referred to as an approximation of the biological system. For example, a one compartment model for a single intravenous dose has five main assumptions. The first is the 'body', in this case the human body, is represented by a single compartment with volume V. Secondly, there is no distribution phase. Thirdly, the single dose is put instantaneously into a single compartment at time zero. Fourthly, unchanged drug is measured in plasma and the plasma concentration is assumed to be C at time t. Finally, loss from the 'body' is assumed to be first order(linear relationship between concentration against time) and the elimination rate constant is represented by k.

Pharmacokinetics models are equations or sets of equations which describe the proposed system. The schematic diagram that is sometimes employed is not a 'model' but rather a pictorial representation from which the appropriate mathematical equation can be derived. Further explanation is given in section 1.5.5

1.5.5 Blood levels of drug at the equilibrium state after multiple dosing

There is ample evidence [Steward et al 1938, De Jongh et al 1948, Van Gemert and Duyff 1950, Burns et al 1953, Wigand et al 1963] that when a fixed dose of drug is administered in fixed multiple-dose regimen, the blood levels of drug will eventually reach a steady state. In this event, the blood level time curve during any dosage interval is the same as it is in the preceding and in the following dosage interval. This equilibrium

state is defined as the condition in which input of drug to the 'body' is equal to output of drug from the body in a given dosage interval.

Wagner et al (1965) derived an equation which can be used to predict the average blood levels during multiple-dose regimen from basic parameters estimated from single dose studies. The equation is;

$$C = \frac{F.D}{Vd.Ke.\tau} \qquad \textit{(Eq. 1.16)}$$

where C is the average blood concentration, F is the fraction of each dose which is absorbed (bioavailability factor), D is the dose(weight) given at the beginning of each dose interval, K_e is the first order rate constant for overall loss of drug from the blood, and V_d is the apparent volume of distribution of the drug. Equation 1.16 will hold under the following conditions, (a) transfer from the blood is first order, (b) F, D, V_d, K_e, and τ are constant for each dose of a multiple-dose regimen in a given patient, and (c) input to the blood and transfer from the blood to any number of possible compartments and back to the blood may be described by a system of simultaneous linear differential equations. However, this equation will be invalidated by several known phenomena (Table 1-6)

i. Carbamazepine, sodium valproate and phenobarbitone

The pharmacokinetic model for the three antiepileptic drugs at steady-state is often depicted basically as one compartmental [Rawlins et al 1975, Kudriakova et al 1992, Yukawa et al 1992, Botha et al 1995a, Bruni et al 1978, Botha et al 1995b] and thus fulfils the above conditions. Based on this, the proposed schematic model is as follows,

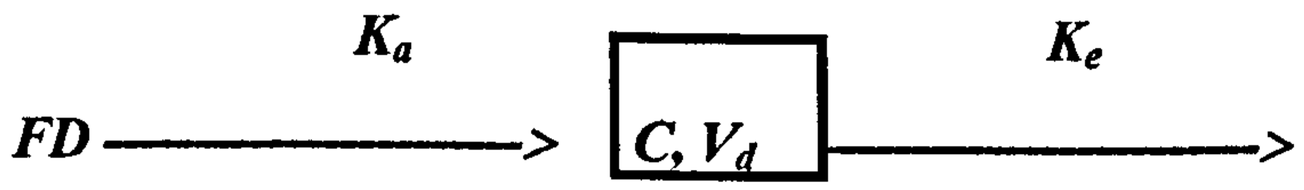


Figure 1-1: The proposed model for carbamazepine, sodium valproate and phenobarbitone at steady-state.

Table 1-6: Phenomena that invalidate equation 1.0

Phenomena	Expected or known result
Plasma level of drug exceeds protein binding capacity	*Ke* increases and *Vd* decreases at high blood levels
Distribution increases at higher blood levels	*Ke* may increase or decrease, and V increases at high blood levels
An enzyme system metabolizing the drug becomes saturated at high blood levels	Order of elimination from the 'body' may change from apparent first order to apparent zero order
The drug's metabolism is stimulated by itself or by another agent	*Ke* increases and *Vd* remains constant
The drug's metabolism is inhibited by another agent	*Ke* decreases and *Vd* remains constant
Diffusion equilibrium is not maintained between drug in blood and drugs in other fluids of distribution	
The fraction of the dose absorbed decreases as the dose is raised due to a low rate of dissolution and relatively fixed transit time in the gastrointestinal tract	*F* will decrease as *D* is increased

Adapted from Wagner et al 1965.

where F is the bioavailability, D is dose, K_a is the absorption rate constant, C is serum concentration, V_d is the volume of distribution, and K_e is the elimination rate constant. In chronic therapy (steady-state), rate of absorption is assumed to equal to rate of elimination. The formula to estimate the relationship of variables such as volume distribution(V_d), apparent elimination rate constant(K_e), and clearance(Cl) from the corresponding serum concentration(C_{ss}) and dose was determined by Wagner et al (1971);

$$C_{ss} = \frac{Dose.\ F}{V_d.\ K_e.\ T} \qquad \textit{(eq. 1.17)}$$

where F is the bioavailability factor and T is the dosage intervals in hours.

However, the relationship between dose and clearance can be estimated as follows;

since $Cl = K_e\ .\ V_d$ *(eq.1.18)*

then $Cl = \frac{Dose.\ F}{Css.\ T}$ *(eq. 1.19)*

ii. Phenytoin

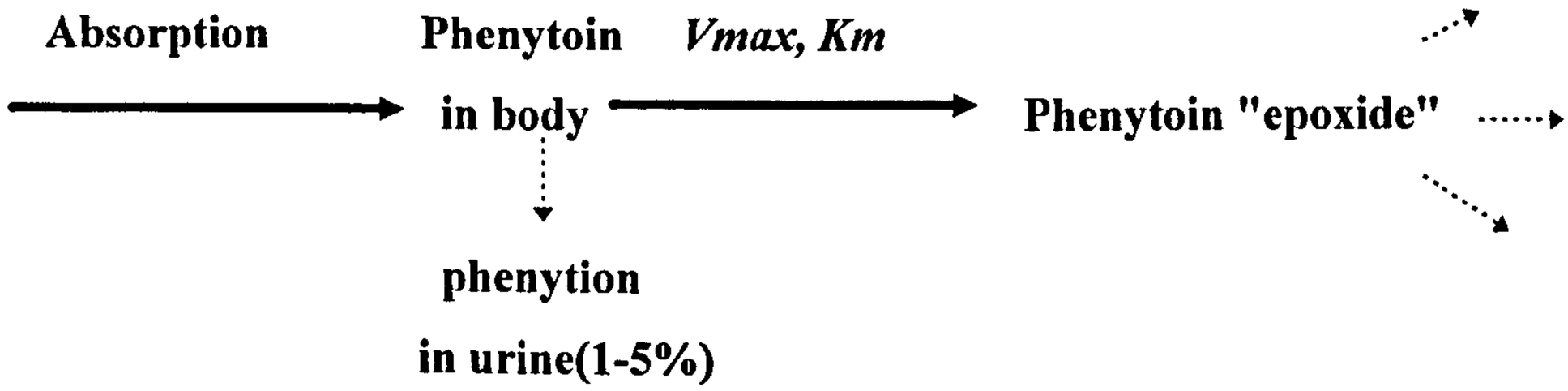
Chemically named as 5-5-diphenyl-2,4-imidazolidinedione is a drug unquestionably difficult to interpret. Three dosage forms are currently available for oral administration. Both phenytoin acid and phenytoin sodium are in tablet or capsule form but, the suspension form is currently available. The important difference between phenytoin acid and phenytoin sodium is the ***salt form factor***(***S***) of 0.92 and 1 [Winter and Tozer 1991] respectively. This factor is important since their bioavailability(F) had been shown to be substantially different. However, Jusko et al (1976) demonstrated that bioavailibility can be almost unity for high quality products.

Phenytoin exhibits a so-called capacity-limited or saturable metabolism [Winter and Tozer 1995] where at near optimum therapeutic concentration there is a disproportionate increase in the plasma concentration as the rate of administration is increased. Figure 1-2 highlights a model for the kinetic behaviour of phenytoin.

Phenytoin elimination appears to be rate-limited by a single metabolic step, presumably to an epoxide intermediate. It is characterized by the Michaelis-Menten enzyme kinetic parameters *Km* and *Vmax*. This phenomenon is assumed to follow

Michaelis-Menten kinetics in which the rate of the metabolism(*v*) is dependent upon the substrate concentration in plasma *Cp*.

Figure 1-2: Pharmacokinetics model associated with phenytoin administration.



where *Vmax* is the maximum rate of metabolism (metabolic capacity) in milligram per day, while *Km* is a constant with a value equal to the plasma concentration at which the rate of metabolism is one-half of the maximum. Some elimination occurs by renal excretion but its contribution is negligible.

$$v = \frac{Vmax \,.\, Cp}{Km + Cp} \qquad \textit{(Eq. 1.20)}$$

Consequently, capacity-limited metabolism is most readily observed at steady-state. This is usually in chronic therapy where the drug is administered at a fixed rate(*R*) until the rate of elimination and the dosing rate(*S.F.R*) are equal.

$$S.F.R = \frac{Vmax \,.\, Css}{Km \,.\, Css} \qquad \textit{(eq.1.21)}$$

where *S* is the salt factor, *F* the bioavailability , *R* is the dose, and *Css* is the serum concentration at steady-state. Equation 1.21 can be rearranged or linearized to;

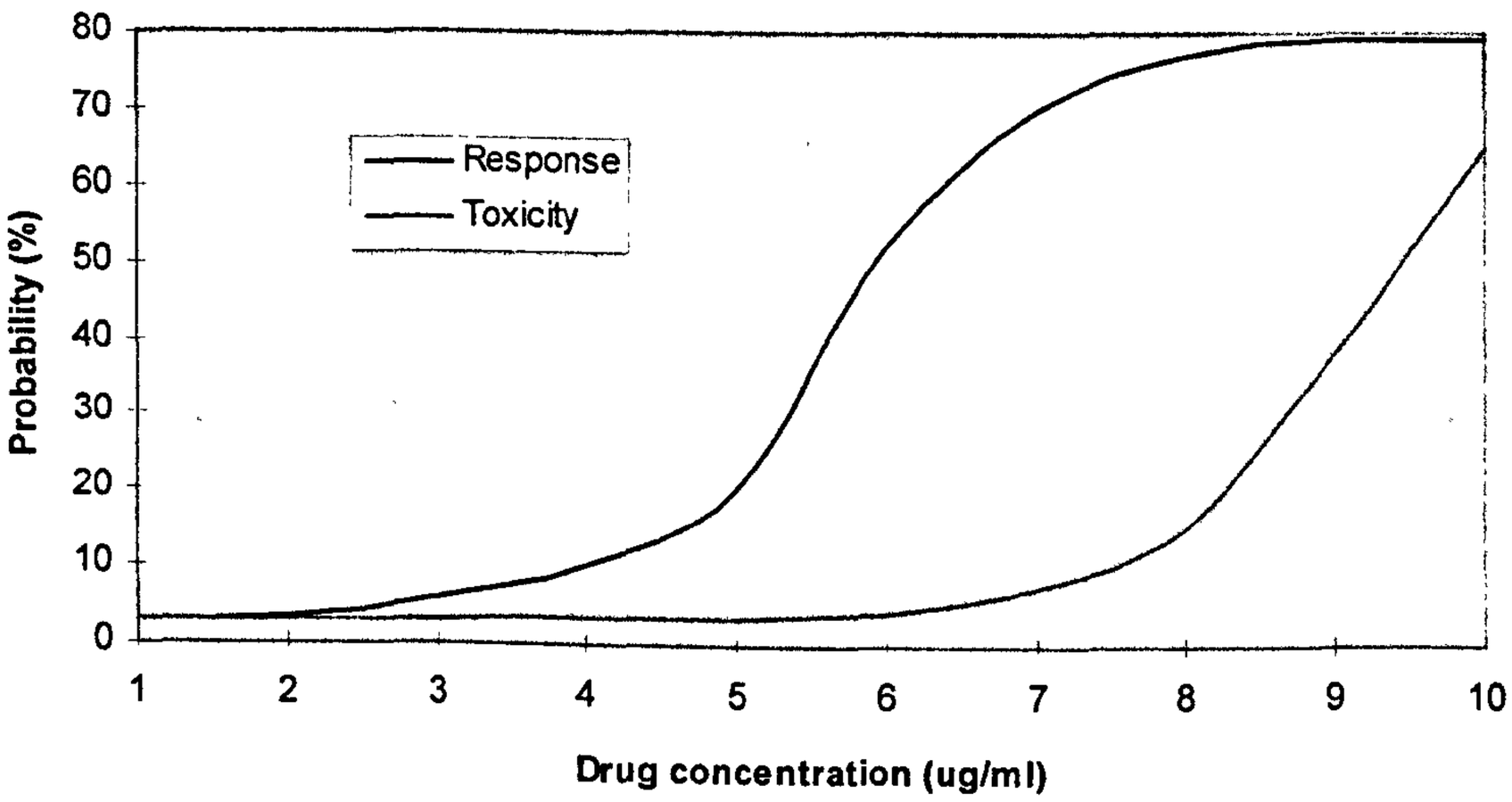
$$S.F.R = Vmax - Km.\frac{S.F.R}{Css} \qquad \textit{(eq.1.22)}$$

where [*S.F.R / Css*] is conventionally called clearance, i.e, it is the parameter that relates the rate of elimination to the plasma concentration. A plot of clearance versus dose would give a straight line with the y-intercept as the *Vmax* and the slope as the *Km*.

1.5.6 What is the "Therapeutic Window" concept?

Most antiepileptic drugs have attributed to them a so-called "*therapeutic range*" or "*therapeutic window*". By definition "*therapeutic range*" is a range of concentrations within which the drug is effective and the probability of unacceptable toxicity is low. Below this range the probability of a positive response is considerably less and above the range there is a considerably increase in the probability of toxicity without any appreciable increase in response [Evans 1991]. Hypothetically, the potential benefits of achieving a drug concentration in the therapeutic range would ensure that the optimum clinical efficacy of the drug can be achieved.

Figure 1-3 : Relation between drug concentration and drug effects for a hypothetical drug



This concept can be depicted graphically in Figure 1-3 for a hypothetical drug. As can be seen, the probability of the desired therapeutic effect is very low (i.e,<5%) when drug concentrations are low (i.e,<5ug/l), as is the probability of toxicity. These reasonable conclusions cannot deny the fact that some small possibility of either the desired response or toxicity even in the absence of a measurable drug concentration. Such incidence would be expected in a large study, assuming that some patients will recover spontaneously without any drug therapy, and that some will develop an adverse effect which is unrelated but coincidental with drug administration. More importantly, as drug

concentration increases between 5 and 10ug/l, the probability of response increases from less than 20% to 75%, then plateaus. Over the same concentration range, the probability of toxicity increases more slowly, from less than 5% to only about 10%, then begins to increase more rapidly as concentrations exceed 7ug/l.

Table 1-7: ***Clinical situations and times in which antiepileptic drug blood level determinations can be valuable***

After starting therapy
Phenytoin (after 2-3 weeks)
Carbamazepine (at 3, 6, 9 weeks)
Valproate (after 3-4 days)
After adding or discontinuing other drugs
Possible toxicity (measure peak level)
Breakthrough seizures (measure trough level)
Suspected compliance

*Adapted from **Mattson RH 1995***

Despite the value of therapeutic drug monitoring in epilepsy, several factors complicate its individual use. They are individual seizure frequency patterns, spectrum of activity of AED, therapeutic range of AED and significant differences in patients' ability to absorb and metabolize these drugs [Welty et al 1983]. However, there are certain clinical situations and times when serum determinations are valuable. Among them is during initiation of therapy and when toxicity is suspected (Table 1-7).

Table 1-8 Therapeutic range and pharmacokinetics data's important during interpreting blood levels of AED

Drugs	Time to staedy state(days)	Target serum levels(mg/L)[a]	Steady-state Half-life (hours)	Absorption (F)-%	Vd (L/kg)	Protein binding(%)
Phenobarbitone	14-21	15-40	100	slow(90-100)	0.6-1.0	48-54
Phenytoin	7-28	10-20	24[b]	slow(85-95)	0.5-0.7	90-93
Carbamazepine	3-4	4-12	24-35[c] 10-15[d]	slow(75-85)	0.8-1.6	70-80
Valproate	2-3	50-120	10	rapid(100)	0.1-0.2	88-92

[a]; Lower or higher concentrations may be optimal for some patients,[b] ;Concentration-dependent
[c];At initiation,[d];Chronic administration
Adapted from :Mattson RH 1995

Individual seizure patterns vary from patient to patient. Those with frequent seizures tend to respond to therapy quicker while patients with seizures at long intervals

may require consistent treatment for a considerable period before the success or failure of a drug regimen is known.

Spectrum of activity for AED is fairly well defined but until now there is no in vitro test for sensitivity of the seizure to a particular drug. Drug therapy is entirely dependent on accurate diagnosis of a seizure disorder by means of the clinical presentation and encephalographic studies [Scheuer and Pedley 1990].

Measurements of total serum levels are however questionable since these drugs are known to be heavily protein bound and the unbound or free drug fraction is the active component. The metabolites of these antiepileptic drugs are also not inert. Some antiepileptic drugs (AED) have metabolites that appear to contribute to therapeutic effect but not routinely monitored. These include the 10,11-epoxide of carbamazepine [Faigle et al 1977] and phenylethylamonamide derived from primidone [Schottelius 1982]. Again, measurement of total, the free form and metabolites are not usually available and are costly to undertake.

The current therapeutic ranges that are widely used for phenytoin, phenobarbitone, carbamazepine and sodium valproate are summarized in table 1-8.. These values are still not absolute and other ranges are also recommended [Welty et al 1983, Shorvon 1995]. Routine serum measurement is controversial because a single serum measurement yields a good approximation of a steady state only for antiepileptic drugs with long half-lives. These include phenytoin and phenobarbitone but those with short half-lives, such as sodium valproate, where random measurement are virtually unpredictable [Loiseau et al 1982] due to the wide fluctuation in concentration over a 24 hour period [Rowan et al 1979].

There are further reasons for doubting the value of AED blood monitoring. The upper limit that is defined as the concentration at which toxic effects are likely to appear but this is most consistent only for phenytoin. But even then some patients can tolerate of serum levels greater than 20ug/ml [Gannaway et al 1981]. The lower limit is difficult to define and many epileptic patients are controlled by serum concentrations well below the optimal range [Turnbull et al 1985]. A study by Dooley et al (1993) further showed discrepancies among paediatric neurologist regarding the upper and lower limits of the therapeutic ranges and the clinical application of levels. They also suggest that both the value and use of antiepileptic drug level need further study.

1.6 Controversial issues about antiepileptic drugs

Although the theories of therapeutic window and therapeutic ranges have been well accepted, complications occur when these concept are implemented across population. As yet, no review has ever been undertaken to confirm the existing findings. Reviews of the therapeutic range for these antiepileptic drugs(AED) are hindered by insufficient clinical trials or limitations of AED studies itself. This has resulted in inconsistent and contradictory conclusion. Thus, Delgado Escueta et al (1983), outlined specific principles in designing clinical trials for AED. Mattson et al (1983) subsequently proposed both selection or inclusion and exclusion criteria in designing prospective evaluation of AED for adults. Looking further, a method of quantification for the evaluation of AED drug therapy was recommended by Cramer et al (1983).

Currently, the uses of these drugs in treatment are highly individualized. However several studies have reported population-based and individual-based pharmacokinetic data for specific patient groups aiming primarily to simplify and improve drug treatments. These pharmacokinetic data were derived using mathematical and statistical computer software. However, population-based and individual-based pharmacokinetics data from a specific group cannot necessarily be used on another group of patients and such application can lead to treatment failure. Although the drugs have long been recognized to display large inter-individual variations, the value of inter-ethnic differences are however rarely studied [Edeki and Brase 1995]. This raised the issue concerning its unknown relationship to ethnicity and possibly the need to have ethnic-based population pharmacokinetics.

Studies on ethnic differences are few and often contradictory. Although most results showed that it is not an important contributory factor, conclusions about the dose and the efficacy of AED are made solely from results obtained from the western population and those of the Japanese community. Dam et al (1977) have indicated that ethnic differences may have significant influence on the plasma clearance of antiepileptic drugs. Peiris et al (1988), found otherwise in that there is no systematic significant difference in pharmacokinetics between Sri Lankan and European population. Unfortunately, they further commented that the Sri Lankan population used were not a representative sample of the epileptic patients population and care should be exercised in generalizing the results.

Studies and reviews on the interethnic variation of metabolism and disposition of phenytoin among different ethnic groups have yet to be quantified. The theory of polymorphism has been attributed to phenytoin and is linked to certain specific genes [Wilkinson et al, 1980]. Essentially phenytoin is metabolized to the glucuronic acid conjugate of the (S)-enantiomer of 5-(4-hydroxyphenyl)-5-phenylhydantoin by the P450 hepatic enzymes, specifically by cytochrome P4502C9 [Veronese et al, 1991, Veronese et al, 1993]. Individuals who have a deficiency of these enzymes are termed as poor metabolizers in contrast to extensive metabolizers who have no enzyme's deficiency. Poor metabolizers are homozygous for an autosomal recessive trait affecting the P4502C9 gene. Studies on familial [Kutt et al 1964, Vasko et al 1980, Vermeij et al 1988] and interethnic differences [Arnold and Gerber 1970, Andoh et al 1980, Kromann et al 1981] distinguished its importance especially in decisions regarding dosing.

Carbamazepine has been known to have autoinduction properties but recent reports showed it too have inter-ethnic differences. These reports were from three Asian countries namely Japan [Yukawa et al 1996], South Korea [Yoon et al 1996] and Taiwan [Lin et al 1991]. However, no similar studies on sodium valproate and phenobarbitone have been reported although these drugs also showed large interindividual dose and blood concentration variation.

The above variations in therapeutic response are linked with the current information above the relevance of heredity to person-to-person differences in response to drugs and environmental substances [Weber WW 1997]. These variations or currently known as pharmacogenetics variations have led to investigators examining the relationship between the metabolic fate of a foreign chemical(drug) in humans and the genetic control of human drug response.

1.7 What is pharmacogenetics ?

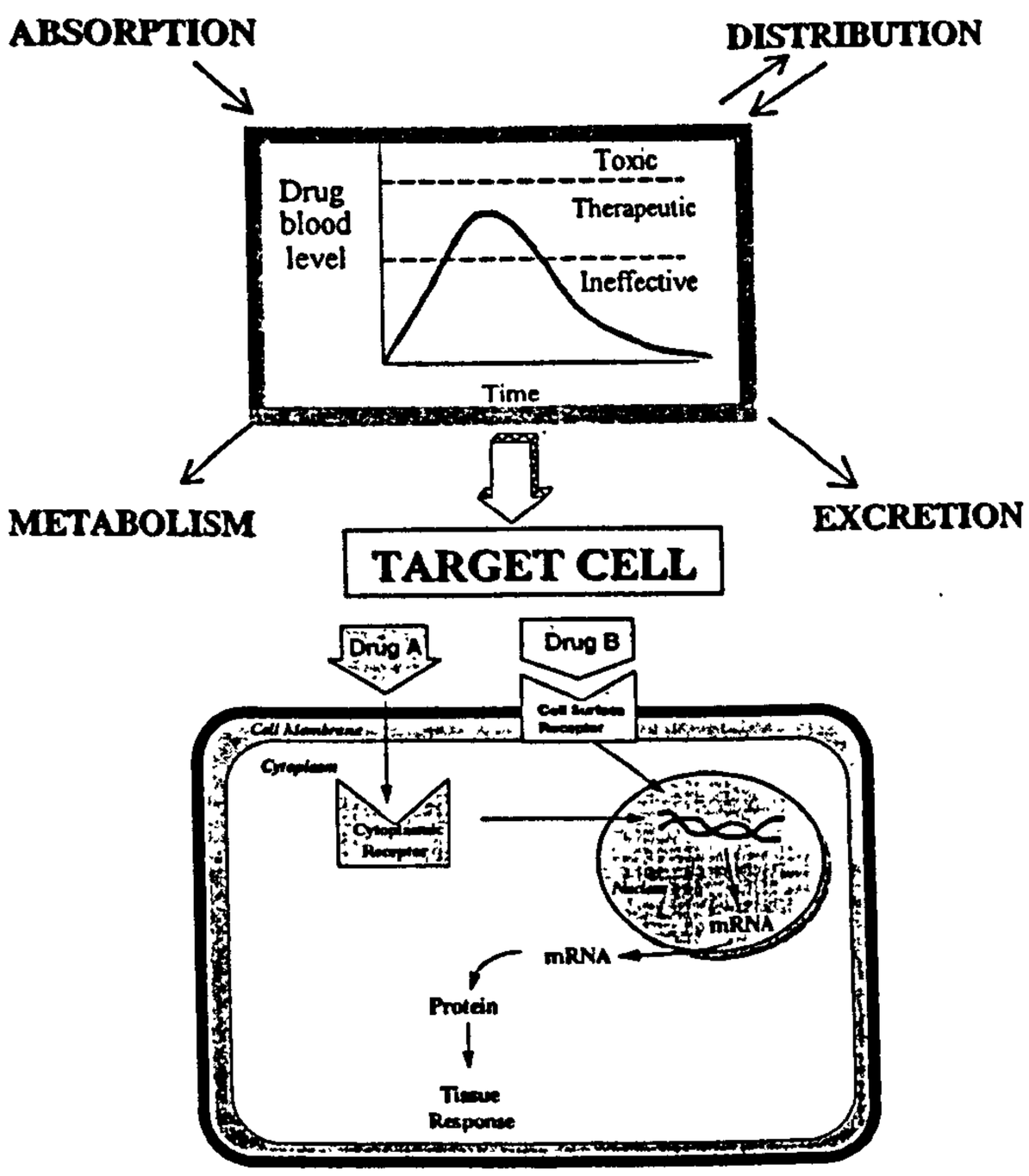
"Pharmacogenetics" studies were started about 40 years ago when early investigators realized the genetics approach to human response to foreign chemicals. With the availibality of modern technology, they managed to separate similar proteins and distinctive patterns of drug metabolites to be identified in different individuals. Pharmacogenetic is then defined by Freidrich Vogel as a study of the effect of heredity on

drug response. It is an experimental science concerned with person-to-person differences in drug response that arise because of the unique genetic constitution of individuals.

1.7.1 Human variation in drug response.

The principles of pharmacokinetics are extensively used in the investigation of the pharmacology and interindividual variability in human drug response. These studies elucidate quantitative relationships between the response of individuals to a given drug and the levels of the drug and its metabolites in fluids and tissues. By doing this, the pharmacological mechanism for the person-to-person variability can be unravelled and can be characterized to a particular pharmacogenetic trait. Mathematical expressions that relate drug concentrations to other standard pharmacokinetic parameters such as dose, dosing interval, bioavailability, rate of elimination, apparent volume of distribution, half-life, and clearance are often adopted to define the relative importance of absorption, distribution, and elimination in the response or collectively known the pharmacokinetics of the drug..

Figure 1-4: Profile of human drug response[adapted from Weber WW 1997]



The pharmacological profile of human drug response can be briefly presented in figure 1-4. Drug response begins the moment the drug is administered and enters the blood stream and ceases when the drug and its metabolites are completely eliminated. As elimination proceeds, three mechanisms that can affect the disposition of the drug to the recipient will occur. First, the drug will move forth and back between the blood stream and fluids and tissues, or it may be transformed if it comes into contact with the enzymes of the liver and other tissues, or a portion of the drug might bind to receptors within or on the cells that result in the characteristic response.

These responses are reversible and short-lived since elimination is essentially complete within a few hours or a few days after exposure to the last dose. Responses may be prolonged if the amount of drug metabolised is small and the drug tends to accumulate in certain tissues such as fat, kidney, lung or bone. In this case, the drug may persist at very low concentration at receptor sites for long periods thereby extending the duration of response.

Table 1-9: Person-to-person variation in plasma elimination half-life values for several agents widely used in medical therapy

Drug	Half-life Variation	Fold variation
Antipyrine	5-15 hours	3
Carbamazepine	18-55 hours	3
Dicumarol	7-74 hours	10
Indomethacin	9-53 hours	6
Isoniazid	0.5-7.5 hours	>10
Rapid acetylators	0.5-1.8 hours	>3
Slow acetylators	1.8-7.5 hours	>4
6-Mercaptopurine	<0.5->1.6 hours	>3
Nortriptyline	15-90 hours	6
Phenylbutazone	1.2-7.3 days	6
Phenytoin	10-42 hours	4
Primidone	3.3-12.5 hours	4
Tolbutamode	4-10 hours	2.5
Warfarin	15-70 hours	>4

1.7.2 Studies on pharmacogenetic- pharmacokinetics diferences.

The extent of intersubject variability in the pharmacokinetics of a given drug is demonstrated in table 1-9. The table shows that three established antiepileptic drugs, namely carbamazepine, phenytoin and primidone(phenobarbitone) have half-lives varying between three and four fold. Pharmacokinetic variability(half-lives) of this magnitude is significantly important since drugs for example phenytoin, which exhibit the so-called saturation kinetics (Michaelis-Menten kinetics), is link to its two common

pharmacokinetics constant (*Vmax* and *Km*). It has been shown that *Km* and *Vmax* can vary to at least 16 fold(4 - 60umol/L) and 10 fold(100 - 1000mg/day) [Garrettson & Jusko 1975, Eadie et al 1976, Mawer et al 1974, Gerber and Wagner 1972, Allen et al 1979]. Similarly, the extent of interindividual variability in carbamazepine and primidone have made establishing the pharmacokinetics of these drugs difficult. Thus, studies on variation of person-to-person (inter-individual) in the pharmacokinetics of antiepileptic drugs are critical in ensuring the desired therapeutic response.

1.7.3 Drug dose-response relationships in pharmacogentics

Drug dose-response relationships depend on the the drug pharmacological mechanisms of response. Currently there are three main mechanisms that are asssociated to interindividual variation. These mechanisms are grouped as pharmacokinetic, pharmacodynamic and those which are classified as unknown and examples are presented in table 1- 10.

Table 1-10: Altered pharmacologic mechanism that contribute to individual variation in human drug response

Pharmacologic mechanism	**Drug**	**Specific pharmacogenetic trait and effect on individual response**
1. Pharmacokinetic mechanism		
-Altered rate of systematic , decreased metabolism	Isoniazid	Rapid acetylation predisposes to therapeutic ineffectiveness
-Altered drug interactions,		
Enzyme inhibation	Phenytoin/isoniazid	Interaction predisposes slowest of the slow acetylator phenotypes to phenytoin toxocity
Enzyme subrate competition	R & S isomers of mephenytoin	
2. Pharmacodynamic mechanism		
-Altered abundance or function of target receptor		
Decreased abundance		
-Altered affinity of receptor	Insulin	Insulin-resistant diabetes mellitus
Decreased affinity	Warfarin, dicumarol	Altered clotting factor receptor predisposes to antocoagulant resistance
3. Pharmacologic mechanism unknown		
-Altered immune response		
Mechanism unknown	Hepatitis B Vaccine	Resistance to hepatitis B vaccine

The relationship between the dose of a drug administered and its response is another important tool to analyze interindividual variability in human drug response. There are two ways to apply this concept in the quantitative analysis of drug effects on biological systems. The first involves the measurement of the intensity of a specified

response relative to the concentration or dose of the drug to produce a graded dose-response relationship. Secondly, the measurement of the frequency of the response relative to the drug concentrations or doses to produce a quantal dose-response relationship. Ideally, the concentration used should be those present at receptor sites, but since these are difficult to measure, the concentration of the drug in blood or plasma or the dosage, is usually substituted. Both these methods can be employed in pharmacogenetic analysis in search of pharmacologic differences between ethnic groups.

1.8 Conclusions

Epilepsy is a disease that has yet to be fully understood. Drug therapy has long been used in treatment and newer antiepileptic drugs are still ineffective for complete seizure control [Pellock 1995]. The complications and difficulty of drug therapy have made almost 33% of epileptic patients suffer unnecessary side-effects and poor therapeutic response either to a single drug or a combination of drugs [Mattson et al 1995, Mattson et al 1990]. Although treatment is complemented by the use of the therapeutic window concept, population-based and individual-based pharmacokinetics and individualized therapy, the search to simplify their use is badly needed. Reports of inter-ethnicity differences have paved the way on research towards the concept of ethnic-specific therapy. The concept of ethnic-specific population-based pharmacokinetics could improve treatment considerably since it can be modelled towards a specific group of people. However, the influence of ethnicity on the efficacy of these therapeutic ranges and pharmacokinetics of established AED's have yet to be confirmed and this thesis will try to assess its importance.

Chapter 2

Objectives

The objectives of this thesis are based on the use of four established antiepileptic drugs, namely phenytoin, carbamazepine, sodium valproate and phenobarbitone in five major government-run hospitals in Malaysia. Studies on these drugs are important due to the relative lack of pharmacokinetic information on Malaysian patients. These drugs are being studied because of three main reasons. The first is their characteristics of having a target concentration range or therapeutic window in optimizing therapy. Secondly, these drugs tend to exhibit high inter and intraindividual variation and finally the concern about reports of inter-ethnic differences in these drugs metabolism and pharmacokinetics. These problems are of clinical importance since the multi-ethnic Malaysian society of Malays, Chinese and Indian, might display differences in handling these drugs and therapeutic responses.

Information regarding the use of these target range and inter-ethnic differences in response is important since the overall treatment outcome may depend on how these factors play its part in the final management of disease. Since Malaysian epileptic patients are predominantly of Malay, Chinese and Indian origin, this study hope to establish the link of the two important characteristics towards drug therapy. Determination on the possible differences would benefit the medical care as a whole and provide invaluable data in countries such as Malaysia where the stress on research and development is currently the main priority. Thus, the main objectives of this study are;

1. to investigate the degree of effectiveness of the present therapeutic range among different ethnic groups.
2. to determine the relationship of dose and serum concentration relationship of carbamazepine, valproic acid and phenobarbitone.
3. to establish the factors(such as age, weight, and ethnicity) which might influence the relationship between dose and serum concentration.
4. to compare the influence of ethnicity on the population pharmacokinetic relationship of these drugs.

Chapter 3

Theories and statistical methods used in studies on population pharmacokinetics of antiepileptic drugs

3.1 Introduction

Pharmacokinetic studies can be categorised into two main types; population-based investigation and individual-based (compartmental or non-compartmental) research studies [Powers 1993]. Population-based studies investigate pooled drug concentrations across more than 1 individual subject. These data are estimated by specially designed computer programmes. Although several computer programs have been tried into practice [OPT by Kelman et al 1982, MULTI-ELS by Yamaoka et al 1987], NONMEM [Sheiner et al 1980] seems to be the most popular to evaluate this information.

Individual-based pharmacokinetic studies require fewer assumptions and involve estimates being calculated for each individual in the study. This requires multiple samples from each patient and is best carried out in the laboratory setting.

Antiepileptic drug population pharmacokinetic values are determined in studies either utilizing the traditional (prospective study design) or routine patient data approaches [Sheiner et al 1977]. These approaches however, can give different and sometimes contradicting findings. Although qualitative reviews have been useful for confirmation, the emergence of systematic reviews which verified findings through quantitative statistical analysis have meant that uncertainties between different findings can be summarised and appraised .

Comparatively, traditional methods such as prospective studies [Henriksen and Johannessen 1982, Mihaly et al 1979] are more controlled and less biased than those from routine patient data. Traditional methods are however restricted by problems of cost, choice of patients and the lack of serendipity. These problems are not encountered in the routine patient data approach and made it more appealing although it has its drawbacks.

The disadvantages of the routine data approach [Botha et al 1995a] include the possibility of bias from concomitant variables that are correlated with included variables, reliability and statistical inefficiency. Several statistical methodologies and computer

software such as NONMEM and MULTI-ELS has shown reasonable accuracy and the problems of data reliability, statistical inefficiency, and methodology can be controlled.

Although reports of pharmacokinetic studies on these drugs are quite common, antiepileptic drug population-based or individual-based studies on specific racial or ethnic groups are however rather limited. Reports and reviews on drug metabolism and disposition [Hvidberg 1986] had shown that antiepileptic drugs such as phenytoin, do exhibit interethnic differences. Statistical methodologies employed in systematic reviews can provide the degree of significance and provide power in outcome estimation and strengthen the use of population-base studies in pharmacotherapy. Thus, this chapter has two main objectives. First, it is aimed to discuss the various methods of estimating individual and population-based studies of antiepileptic drugs. Secondly, to highlight the importance of employing statistical methods commonly used in systematic review to population based studies in order to solve unanswered questions especially in the field of medicine.

3.2 Population-based studies

The current interest in determining the population characteristics of pharmacokinetic parameters describing drug metabolism and disposition have led to the great deal of experimental work directed towards it. Population based studies characteristically include three main objectives. The first is to estimate the mean values of parameters, their quantitative relationship to individual physiology and finally their variability across population [Sheiner et al 1977]. These are particularly important for optimal design of dosage regimens for individual patients and to provide information into the mechanism by which drugs are absorbed and eliminated.

The clinical use of pharmacokinetic methods for individualizing drug therapy is initiated with the aid of population pharmacokinetic data. A knowledge of population pharmacokinetics provides the basis for the initial drug regimen. Usually, one or just a few drug concentrations taken from the patient and interpreted in the context of the population data enable one to estimate individualized pharmacokinetics which may lead to further refinement of the dosage regimen.

There are two requirements for applying population data to the individual:

i. a relevant population pharmacokinetic data base

ii. a framework for linking patient to the population.

Population pharmacokinetics thus entails the summarization of pharmacokinetic studies in groups of patients and the establishment of relationships between individual patient characteristics and pharmacokinetic parameters.

3.2.1 Statistical methods employed in population-based studies

Pharmacokinetic parameters such as bioavailability, volume of distribution and clearance, have traditionally been estimated by studies of drug disposition in a group of homogenous patients. This value is typically termed as population-typical value. This is usually in the form of mean or average values. The extent to which these values differ from the population is estimated by measuring the interindividual deviation and is term as population-variability value. The mean and standard deviation thus summarize the population distribution of pharmacokinetic parameters. The mean and standard deviation for a "normal" distribution may be interpreted parametrically, i.e, the mean is located in the center of the normal distribution and the ± one standard deviation accounts for 68% of population values [Hannagan 1986]. For a skewed distribution, the log of the parameter often transforms the distribution to a normal curve [Gabrielsson and Weiner 1997].

Findings from population based studies described above suffer from inadequacy due to restriction in study design. These results are not estimates of 'real' population. Thus, the Sheiner & Grasela (1991) mixed effect model technique of using information generated during routine patient care to estimate population pharmacokinetics should provide an alternative in generating results from wide group of individuals. The individuals should exhibit a range of patients' characteristics that are thought to influence drug disposition. These characteristics should include a group of patients with varying disease states, weight, ages, and certain important laboratory tests. This is done deliberately so as to establish relationships between individual patient characteristics and population pharmacokinetic parameter distributions. The uncovered relationships may be

categorically quantitative as in the observation that smokers tend to have 50% to 60% higher theophylline clearance values than non-smokers[Matsunga et al 1989].

Hashimoto and Sheiner (1991) have compared the population approach with the individual-based approach, along with the advantages of having population pharmacokinetic data available to estimate pharmacodynamic parameters. Their conclusions were;

i. Even small amounts of pharmacokinetic data, (even if) suboptimally sampled, can markedly improve population pharmacodynamic parameter estimates when the analysis model is correctly specified.

The conclusion was however unclear since there was no mention of reasons as to why a small amount of sample is adequate to specified pharmacokinetic data study designs.

ii. In general, for both pharmacokinetic and pharmacodynamic data, designs involving more individuals, even if many are sparsely sampled, dominate designs calling for a more complete study of fewer persons.

iii. A full population analysis which combines pharmacokinetic and pharmacodynamic investigation, generally deals with sparse data more flexibly than individual-based analysis. When the model is mis-specified, then a population analysis emerges as more powerful than an individual based one.

iv. Randomising times of sampling for sparse pharmacokinetic data designs in general does no harm, and , when the model is misspecified, provides considerable protection. Randomising dosages adds useful information and also protects against model misspecification.

Other benefits of the population based approach also require fewer design criteria and are adaptable to the clinical setting, i.e subjects can be patients currently being treated with the drug under consideration. Another distinct advantage is the flexibility of sampling times and the capability of the clinician to use information from the critically ill, the geriatric patient or even from the very young. These patients would not be available for an individual-based type of study because of the relatively large number of patients needed [Powers JD 1993].

3.2.2 Data analysis methods

Jelliffe et al 1998 summarises population pharmacokinetic models into three main categories. These are parametric population pharmacokinetic models[Rowland et al 1985], semi-nonparametric and hierarchical Bayesian population models [Wakefield et al 1994 and Davidian and Gallant 1993] and the nonparametric population models [Mallet 1986 and Schumitzky 1991]. Parametric population modelling methods involved estimating means and SD's of the pharmacokinetic parameters, and correlation (or covariances) between them. Semi-nonparametric and hierarchical Bayesian population models follow the Bayesian strategy of sampling and Bayesian inference about a population. Non-parametric population methodology is actually a discrete(not continuous), spiky (not smooth) probability distribution in which no preconceived parametric assumptions (such as lognormal) have to be made about its shape (Mallet 1986).

Basically, the three common methods of population-based pharmacokinetic studies of antiepileptic drugs employ the parametric population pharmacokinetic models. The first is called the naive pooled data approach, second, the standard two-stage approach, and finally the NONMEM approach. NONMEM is an acronym for analysis of a non-linear mixed effect model [Sheiner and Beal 1980]. The first method was proposed to be abandoned due to inaccuracy and the availability of more precise and robust methodology. Two-stage and NONMEM approaches applies parametric strategies in pharmacokinetic modelling and NONMEM have since been the most widely used population pharmacokinetic computer software in antiepileptic drug studies.

The limited use of the naive pooled data approach is mainly because of the need of an accurate and consistent estimation of both population-based and individualised-based pharmacokinetic parameters. The naive pooled data approach lacks this ability since the method analyzes all data as though it all come from one individual. If, taking as an example, of the famous Michealis-Menten equation as the pharmacokinetic model,

$$R_{ij} = \frac{Vm_j \,.Cpss_{ij}}{(Km_j + Cpss_{ij})} \qquad \textit{(eqn.3.0)}$$

$$\bar{R}ij = Rij + \varepsilon_{ij} \qquad \textit{(eqn.3.1)}$$

where R_{ij} is the weight adjusted dosage rate(mg/kg/day) for the ith pair in the jth patient; Vm_j is the maximum elimination rate for the jth patient, $Cpss_{ij}$ is the steady-state plasma concentration(ug/ml) measured in the jth patient, and Km_j is the Michealis-Menten constant for metabolism in the jth patient.

Since the naive approach analyses all patients as one single individual, the j disappear from the equation 3.0 and 3.1 and the subscript i indexes all $\bar{R}$-$Cpss$ pairs. Although this method can estimate for population parameters such as Vm and Km and the corresponding confidence interval, no estimation of the standard errors(ε_{ij}) for Vm and Km are obtained. This method thus measures variability from all sources with no effort to determine either intraindividual and interindividual variation. The NONLIN computer software for nonlinear least square approach [Metzler et al 1974] provide actual data fitting for this method.

The naive pooled data approach employs regression technique in population-based studies. Regression objectively attempts to show the relationship between two variables by providing a mean line that best indicates the trend of the points or coordinates on a graph. The slope indicates the rate of change in one variable against the other and regression shows this slope. Correlation coefficients do not provide any information on the slope of the line between two variables.

The equation that describes a linear regression line is usually in the form of $y = \alpha + \beta x$, where α is the intercept and is the β regression coefficient. Both α and β are used as population parameters. The regression equation is an example of what is termed a model with which one attempts to model or describe the relationship between y and x. On a graph, α is the value of the equation when $x = 0$ and β is the slope of the line. The method of calculating of this relationship is called the method of least squares.

Since α and β are characteristics of a population obtained from estimates from a sample of a population, the estimates are labelled as a and b. The estimates of a and b for any subject i, x_i and y_i can be predicted from the estimated regression equation of $Y_i = a + b\ x_i$, where Y_i is called the residual sum of squares. The regression line is thus drawn through the n points on the scatter diagram so as to minimise the sum of squares of the distances, $y_i - Y_i$, of the points from the line, these distances being measured parallel to the y-axis. This then leads to the value of a and b being called the least square estimates of the

population parameters of α and β. The model is now $y_i = \alpha + \beta x_i + \varepsilon_i = Y_i + \varepsilon_i$, where ε_i are the amount the observed value differs from the predicted from the model and is assumed to be normal with the average being equal to zero.

A linear regression line fitted by least squares can be typically demonstrated by the following presentation. The straight line equations ; y = $a + bx$, where y and x are two variables, a represents the intercept, and b represents the slope or gradient of the line.The equations for finding the slope and the intercept are:

$$b = \frac{(\Sigma xy - \frac{\Sigma x \ \Sigma y}{n})}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \qquad \textit{(eqn 3.2)}$$

or

$$b = \frac{(n\Sigma xy - \Sigma x \Sigma y)}{n\Sigma x^2 - (\Sigma x)^2} \qquad \textit{(eqn 3.3)}$$

and $a = \bar{y} - b\ \bar{x}$, where $\bar{y}$ and $\bar{x}$ are the mean of the two variables. This can be presented by the following simple example ;

Example 1. The Least square method

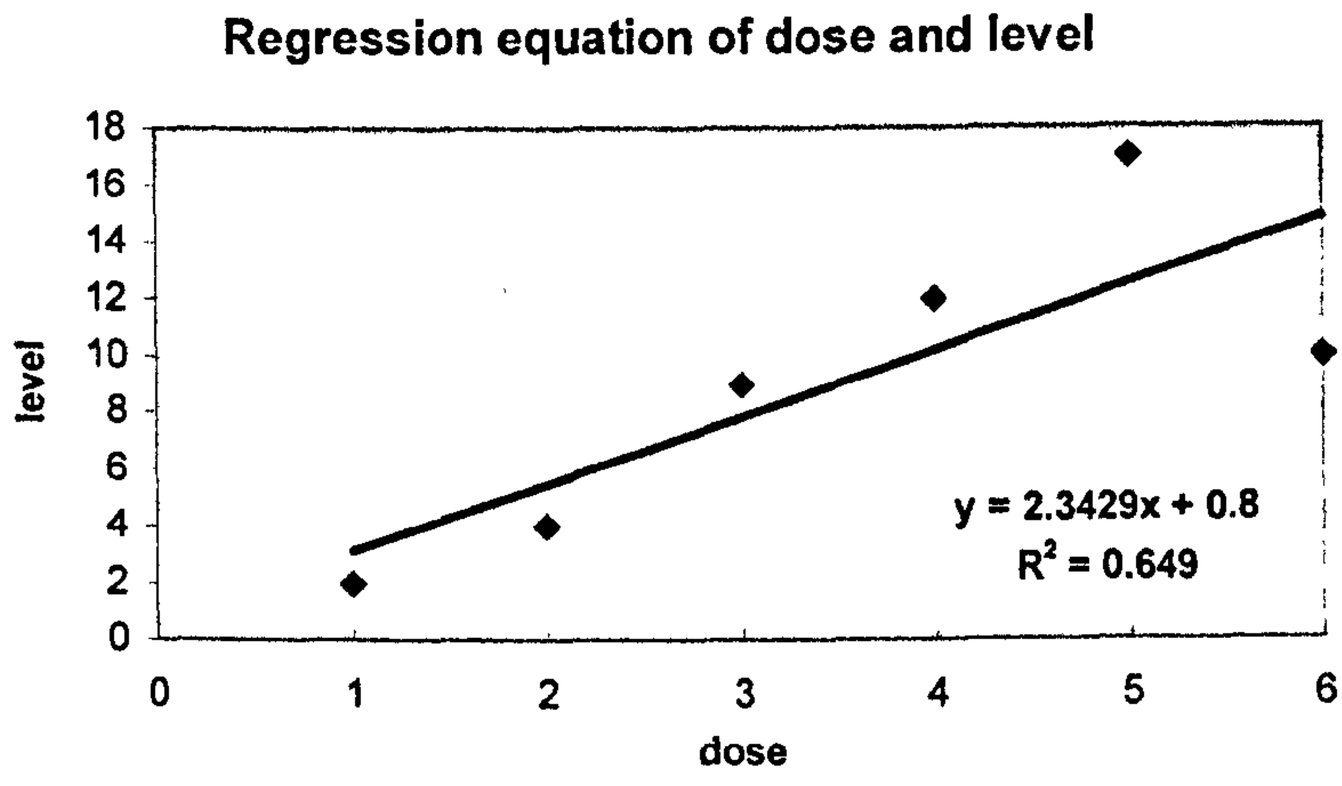
No.	Dose(x)	Level(y)	x2	y2	xy
1	1	2	1	4	2
2	2	4	4	16	8
3	3	9	9	81	27
4	4	12	16	144	48
5	5	17	25	289	85
6	6	10	36	100	60
sum(Σ)	21	54	91	634	230

$\bar{x} = 21/6 = 3.5$ $\qquad$ $\bar{y} = 54/6 = 9$,

$$b = \frac{230 - (21 \times 54)/6}{91 - (21 \times 21)/6} = 2.34$$

since $a = \bar{y} + b.\ \bar{x}$, then , $a = 9 - 2.34(3.5) = 0.81$

The equation of the regression line is $y = 0.81 + 2.34\ (x)$, and graphically shown as below.



It should be noted that the least square regression technique is limited to linear relationship between the variables y and x. As stated earlier(chapter 1), most first order models are non-linear, thus transformation by logarithm of data which is crucial for linearization(normal distribution) of the model.

The two stage approach uses each individual patient dose and serum level pairs to calculate the parameter estimate. This method does account for intraindividual and interindividual variation where the statistical errors(ε_{ij}) of parameters are obtained from the residuals of regression. The use of lognormal treatments are not only to transform the exponential equation into linearized equations but also to account for any intraindividual and interindividual errors in the real world.

Intraindividual error is meant to represent all uncertainty caused by intraindividual time variation in Km_j and Vm_j, pharmacokinetic model misspecification, analytical error in $Cpss_{ij}$ and reporting error in R^o_{ij}. Interindividual error encompassess all differences between individuals such as genetic and ethnic background. Both intraindividual and interindividual errors are independent and identically distributed with mean zero and variance equal to σ^2.

Estimation of parameters are obtained from two or more sets of data from each individual and the arithmetic average is chosen as the estimated population parameters.

The estimate of variance(σ_ε) for the population is taken to be the square root of the sum of the individual residual sums of squares over all individuals, divided by the total residual degrees of freedom. The estimate of variance for *Vm* (σ_{Vm}) and *Km* (σ_{Km}) is then estimated by the sample standard deviation of each individual. This method is however has its limitation in that no confidence intervals or standard errors for estimates(σ_{Vm} and σ_{Km}) can be calculated.

The NONMEM approach describes by Sheiner and Beal (1980) is currently the only method that takes into account the weaknesses of the above methods. The method employed the extended least squares approach which is actually the maximum likelihood approach and being used widely for all four drugs. Its objective function assumes uncorrelated statistical errors having constant variance, where the standard errors and their 95% confidence levels are easily computed.

ii. Curvilinear relationship.

The straight line equation relationship mentioned above is a good empirical statement of the relationship between two variables even when the true relationship between two variables is more complex than the straight line portrayed. It may be just as important to know the exact or approximate "form" of the relationship as it is to have an empirical statement of it. For this reason, it is necessary to consider other ways of expressing a relationship than a straight line.

Other ways to represent relationships are by curves. The equations of a number of curves which can be useful in statistical analysis of the relationship between variables are;

$$Y = a + bX + cX^2 \qquad \textit{(eqn 3.4)}$$

$$Log\ Y = a + bX \qquad \textit{(eqn 3.5)}$$

$$Log\ Y = a + b\ log\ X \qquad \textit{(eqn 3.6)}$$

$$Y = a + b\ log\ X \qquad \textit{(eqn 3.7)}$$

$$Y = 1/(a + bX) \qquad \textit{(eqn 3.8)}$$

$$Y = a + bX + cX^2 + dX^3 \qquad \textit{(eqn 3.9)}$$

$$Y = a + bX + c(1/X) \qquad \textit{(eqn 3.10)}$$

Figure 3-1: Curves illustrating a number of different types of mathematical functions[adapted from Ezekiel & Fox (1965)]

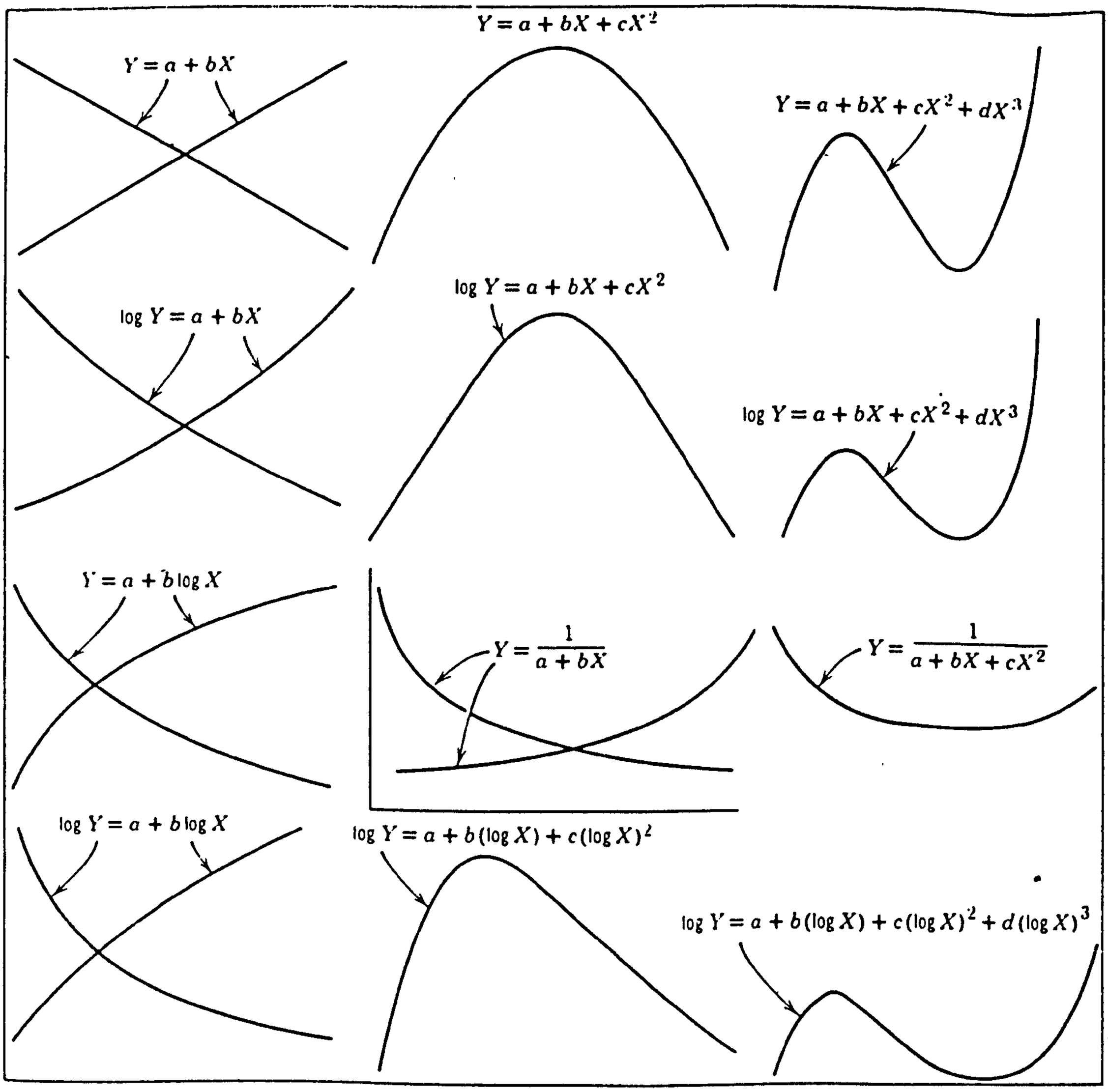


Figure 3-1 illustrates the different of type of curve for each of the above equations. The diversity in the shape of the different curves is striking and the differences in the shapes depend solely on the particular form of equation used to compute them. There are certain types of relationship that can be accurately represented by each of these equations. When it is "fitted" to data where that type of relation is really present, it can give a curve that accurately represents the central tendency of the data. However, if the same equation is fitted to data for which the underlying relationship follows a different function, the resulting curve gives a distorted representation of the true relation. Thus, the representation of each equation only shows the relation in so far as it is possible to do so within a particular limit.

3.2.3 Limitation in estimating one variable from one known values of another

The limitations in estimating one known variable from known values of another can be verified by examining the "closeness" with which the line or curve fits the original data. This then led to the importance of measuring accuracy of estimate and the degree of correlation.

i. Correlation

When both x and y are random variables, an index called product-moment correlation or simply correlation coefficient may be useful to measure the extent the relationship between them. *Correlation coefficient (r)* can be defined by

$$r = \frac{\Sigma[(x - \bar{x})(y - \bar{y})]}{\sqrt{[\Sigma(x - \bar{x})^2 \Sigma(y - \bar{y})^2]}} \qquad \textit{(eqn 3.11)}$$

For any set of data, r falls within the range of -1 to +1. r will equal to one if both variables increases together, while r will be -1 if one variable decreases as the other increases.

The *squared correlation coefficient (r-squared)* is the fraction by which the sum of squares of y is reduced to give the sum of squares of deviations from the regression on x. Similarly r-squares values will range from $0 \leq r^2 \leq 1$.

i. Sampling errors in regression and correlation

The above calculations take into consideration that in the whole population the mean value of y does not change with x, i.e the null hypothesis should be $\beta = 0$. To test the hypothesis, the t distribution test on n-2DF, is employed.

$$t = \frac{b}{SE(b)} \qquad \textit{(eqn 3.12)}$$

where $b = \beta$, $SE(b)$ is the square root of the variance of b or alternatively

$$t = r\sqrt{[(n-2)/(1-r^2)]} \qquad \textit{(eqn 3.13)}$$

Confidence limits for β or b at 95% level can be obtained as

$$b \pm t_{n-2,0.05}\,SE(b) \qquad \textit{(eqn 3.14)}$$

The 95% confidence limits for the predicted value of y at a any value of x, eg. x_o is given by

$$Y \pm t_{n-2,0.05}\, s_o \sqrt{\left[1/n + (x_o - \bar{x})^2 / \Sigma(x - \bar{x})^2\right]} \qquad \textit{(eqn 3.15)}$$

where S_o is the residual mean. The width of the confidence interval increases with $(x_o - \bar{x})^2$, and therefore a minimum when $x_o = \bar{x}$. Reason for the increase in width of the interval is any slight sampling errors of b will have a greater effect for values of x_o distant from $\bar{x}$ than for those near $\bar{x}$.

3.2.4 Multiple regression

In multiple regression, each individual provides observations simultaneously on several variables which then means that the data are multivariate. Thus, in order to explore the relationship of one variable to more than one other variable, the method of multiple regression is employed. This approach expresses the mean value of the dependent variable in terms of a set of other variables, usually termed as *independent variables, predictors, explanatory variables or covariates*. Example of these independent variables could be

age, height or arm length. The regression equation can be satisfied if all the variables are given in their mean values. Thus,

$$y = a + b_1 x_1 + b_2 x_2 + \ldots\ldots\ldots + b_p x_p \qquad \textit{(eqn 3.18)}$$

where b_1, b_2, *and* b_p are called *partial regression coefficients*, a is the intercept. x_1, x_2, *and* x_p are the predictor variables. The number of predictor variables, p, should be less than the number of observations, n, and the same p predictor variables must be available for each individual in any one analysis.

By utilizing multivariate regression equations, the final result serves to sum up all the evidence of a large number of observations in a single statement. This equation expresses in a condensed form the extent to which differences in the dependent variable tend to be associated with differences in each of the other variables.

3.3 Methods for estimating measurement

Population outcome measurement can be analysed by the use of methods employed in meta-analysis. The common analytic theme expressed in techniques for combining results across studies is the weighted average of study-specific results. Among the statistical methods that are commonly used are those by Mantel and Haenszel (1959) and Yusuf et al (1985) (Peto Method), which use the odd-ratios to deal with variability among study results. Characteristically, both these methods are very similar and are based on a fixed effect model and an explanation for the Mantel-Haenzel method is presented in the following example.

Arrangement of data and table notation for application of Mantel-Haenzel and Petos methods.

	Treated	Not treated	Total
Diseased	*ai*	*bi*	*gi*
Not diseased	*ci*	*di*	*hi*
Total	*ei*	*fi*	*ni*

where *ai, bi, ci, di* are the number of patients treated and not treated in either disease or non-disease group. *gi* is the total of $ai + bi$, $hi = ci + di$, $ei = ai + ci$ and finally $fi = bi + di$.

The summary odds ratio of Mantel Haenzel(ORmh) can be calculated by the formula

$$OR_{mh} = \frac{\text{sum}(\text{weight}_i \text{ X } OR_i)}{\text{sum weight}_i}$$

where $$OR_i = \frac{ai \, x \, di}{bi \, x \, ci}$$

while $\text{weight}_i(w_i) = 1/(\text{variance}_i)$

and $\text{variance}_i = ni/(bi \, x \, ci)$

95% confidence interval(C.I)

$$95\% \text{ C.I} = e^{\ln ORmh \pm 1.96 \sqrt{(\text{variance ORmh})}}$$

where variance ORmh is calculated by the method of Robins, Greenland and Breslow 1986.

$$\text{VarianceORmh} = \frac{\sum F}{2\sum R^2} + \frac{\sum G}{2 \, x \sum R \, x \sum S} + \frac{\sum H}{2\sum S^2}$$

where $F = ai \, x \, di \, x \, [(ai + di)/ni^2]$

$$G = \frac{[ai \, x \, di \, x \, (bi + ci) + (bi \, x \, ci \, x \, (ai + di)]}{ni^2}$$

$$H = [bi \, x \, ci \, x \, (bi + ci)]/ni^2$$

$$R = [ai \, x \, di]/ni$$

$$S = [bi \, x \, ci]/ni$$

Regression methods have also been used by Steinberg et al (1991) but Longnecker et al 1988 used this technique to estimate the slope of the dose-response relationship between alcohol and breast cancer adjusting for study quality. The combination of means or mean difference across studies is also possible and sometimes appropriate [Fleiss 1986].

The methods described above employed the so-called "**fixed effects**" models, which assume the existence of a single effect of exposure common to all studies. The among-study variability in summarizing relative risks and ratios is thus ignored. DerSimonian and Laird (1986) proposed the "**random effect**" model. In this case, the

existing studies are viewed as a random sample of a population of studies and treat among-study variability as an integral part of the data.

Population outcome measurement can be from primary or secondary data. Primary data are data from true experiments either prospective or retrospective. However, prospective data used in meta-analysis are usually gathered from published or unpublished literature. This involves an intensive search of all literatures through the electronic databases such as the Medline or BIDS, and using specific key words and the percentage of recovery is between 80 - 90% [Dickersin et al 1994]. Currently, evidence based pharmacotherapy had been using this method extensively for verification of many drug-disease problems [Mumford and Dam 1989, Piccinelli et al 1995, Marson et al 1996, Marson et al 1997].

Primary data from routinely monitored patient's drug serum concentration have been used in estimating population pharmacokinetics in phenytoin [Sheiner and Beal 1980], carbamazepine [Delgado Iribarnegaray et al 1997], sodium valproate [Botha et al 1995b] and phenobarbitone [Yukawa 1991]. The main component of the analysis is to obtain a summary estimate of outcome measures and to test for homogeneity across both variables and covariables.

Heterogeneity is a statistical phenomena when a sampling variation is unlikely to explain two groups of incidences. This can be due to the trials characteristic are different and analysis on the confounding variables needs to be explored. Thus, the following methods are recommended.

3.3.1 Stratified analysis

This method employed the fixed effect model and is effective either to include and exclude any confounding factors involved in the analysis. Fixed effect model is validated by the following assumption: a) summary effect estimates(θ_s) which is calculated from each of the individual estimate(θ_i), satisfies the distributional relationship $\theta_s \sim N(\theta_i, w_i^{-1})$ where θ_i is the individual estimate in the ith study. w_i^{-1} is the asymptotic variance (reciprocal of weight) of θ_s. b) the weighted summary estimates($w_i\theta_s$) satisfies the distributional relationship of $w_i\theta_s \sim N(w_i\theta_i, w_i^{-1})$ with the null hypothesis of $H_{oi} : \theta_i = 0$, $w_i\theta_i \sim N_i(0, w_i)$.

The procedures include grouping each study into different groups according to the possible confounders (variables) such as sex, age, race etc and analyzing each group separately. The method although simple is restricted as only one or two confounders can be investigated [Kleinbaum 1982].

3.3.2 Mixed effect regression analysis

Both of the above methods deal on a fixed effects model. However, when heterogeneity across the study still remains unexplained, the mixed effect regression analysis should be considered. This method is based on turning the results from a fixed effect model into the random effect model where the variations among studies are accounted for and a weighted average effect is estimated. It is in fact incorporating the meta-regression analysis into the random effects model [Huque 1994] by decreasing between study variance.

This difference between fixed and random effects can be distinguished by the following analogy. Let supposed the effect estimate θ_s of the fixed effect model that satisfies the distributional relationship $\theta_s \sim N(\theta_i, w_i^{-1})$, is now $\theta_i \sim N(\theta, \tau^2)$, where the observational effect from each study is independent from $N(\theta, \tau^2)$[DerSimonian & Laird 1986] and τ^2 is the asymptotic variance for each study. This assumption theoretically allows heterogeneity across studies by following the marginal distribution of θ_s, that is, $\theta_s \sim N(\theta, w_i + \tau^2) = \theta_s \sim N(\theta, (w_i^*)^{-1})$. The formula to estimate $(w_i^*)^{-1}$ and the steps to undertake the random effect model is as follows;

1. Step 1

The effect estimate of θ_s can be calculated from the equation given by Whitehead (1991),

$$\theta_s = \Sigma w_i^* \theta_I / \Sigma w_i^* \qquad \textit{(eqn 3.19)}$$

Step 2.

Calculate the statistic*(U)*

$$U = (\Sigma w_i^* \theta_I)^2 / \Sigma w_i^* \qquad \textit{(eqn 3.20)}$$

where it follows a chi-square distribution with 1 degree of freedom under the null hypothesis, Ho: $\theta = 0$.

Step. 3

The corresponding approximate 95% confidence interval is

$$\theta_s \pm 1.96 \sqrt{(1/ w_i^*)} \qquad \textit{(eqn 3.21)}$$

where $w_i^* = (w_i^{-1} + \tau^2)^{-1}$, τ^2 is estimated by DerSimonian & Laird (1986), while τ^2 is calculated by;

$$\tau^2 = \frac{max[0, Q - (k - 1)]}{\Sigma w_i - (\Sigma w_i^2) / \Sigma w_i} \qquad \textit{(eqn 3.22)}$$

if $\tau^2 = 0$, $w_i^* = w_i$, the random effects model will produces a result similar to the fixed effects model. This will then indicate homogeneity across studies.

Homogeneity test is similar to fixed effect model, using the Q statistic, i.e

$$Q = \Sigma w_i (\theta_i - \theta_s)^2 \qquad \textit{(eqn 3.23)}$$

which is a weighted sum of squares of deviation. If the treatment effects are homogenous, Q follows a chi-square distribution with (k - 1) degrees of freedom. [Cochran 1954].

To demonstrate the random effect model, the following example is illustrated.

Study	θ_i	w_i	w_i^*
1	0.5	30	7.5
2	1.0	40	8
3	1.5	10	5

For the fixed effect model, the calculation yield

$\Sigma w_i = 80$, $\Sigma w_i^2 = 2600$, $\Sigma w_i . \theta_i = 70$, $\Sigma w_i . \theta_i^2 = 70$,

and the summary effect estimate (θ_s),

θ_s = 70/80 = 0.9

Confidence interval(C.I), 95% = 0.7, 1.1

U statistic = 4900/80 = 61.5

Test of homogeneity(Q) = $70 - (0.9)^2 . 80$

= 5.2 ($p > 0.05$)

The fixed effect test statistic U showed a highly significant relationship. The test for homogeneity also show there is homogeneity across studies.

If the above data are to follow the random effect model, then the calculation is as follows,

$$\tau^2 = \frac{(5.2 - 2)}{(80 - 2600/80)}$$

$$= 0.1$$

$$\Sigma w_i^* = 20.5$$

$$\Sigma \theta_1 . w_i^* = 19.3$$

$$\theta_s = 20.5 / 19.3$$

$$= 1.1$$

$$\text{U* statistic} = 134.7 / 20.5$$

$$= 6.6 (p < 0.05)$$

$$\text{95\%,Confidence interval} = -0.1, 2.3$$

The results not only showed a wider confidence interval than that of the fixed effect model but also showed that the summary effect is heterogenous. The result higlighted that the summary effect is affected by variability between studies.

3.3.3 Statistical issues in meta-analysis

Studies that measure effects on a continuous scale are often subjected to meta-analysis. Meta-analysis is important since it is able to estimate an overall measure of effect and the relationship between disease and some measure of intensity of exposure. The use of statistical models in meta-analysis is however useful in examining the reasons for heterogeneity or to explore the relationship between various study description and effect size. There are methods that are not recommended and these issues will be addressed and discussed.

a. Effect size

i. ***Outcome measure of same scale***

Cochran 1954 described the methods to combine results from different experiment using the analysis of variance analogy. Both the fixed and random effect models were described extensively and it was its application towards of mixed effect models was also possible. Yudkin et al 1991 duly used this method in describing the change in Kutzke disability status scale at two years in four randomized trials of the effect of azathioprine treatment in multiple sclerosis.

ii. ***Effect size of different scales***

This is a method where summary estimate of studies with different scales to measure effect can be calculated. This method requires that an estimate of effect size for each study must be made into a common metric. Hedges (1982) provided the formula to estimate a summary effect size for studies that compare two groups taking into account the size of the two groups;

$$d_s = \frac{\Sigma(w_i \cdot d_i)}{\Sigma w_i} \qquad \textit{(eqn 3.24)}$$

where d_s is the summary estimate of the difference in the effect size measured in a common metric, w_i is the weight assigned to each study, and d_i is the effect size.

$$w_i = \frac{1}{variance_i} \qquad \textit{(eqn 3.25)}$$

where

$$d_i = \frac{(mean_e - mean_c)}{Sd_{pi}} \qquad \textit{(eqn 3.26)}$$

where d_i is the common metric that measures effect size in the ith study, $mean_e$ is the mean in the experimental (or exposed group), $mean_c$ is the mean in the control (or unexposed) group, and SD_{pi} is the pooled estimate of the standard deviation of the effect measure for each study. When the study involves a before-after comparison, $mean_e$ and $mean_c$ are the mean differences.

iii. ***Test of homogeneity***

Both the above methods can be tested for homogeneity using the Q statistic [Cochran 1954]. Q referred to the chi-square distribution is calculated and used to test the

hypothesis of homogeneity. If there is no statistical evidence of lack of homogeneity, a 95% confidence limit for the summary estimate of effect is calculated. The formula to test homogeneity is describe as follows;

$$Q = \Sigma(weight(w_i) . (mean_s - mean_i)^2) \qquad (eqn\ 3.26)$$

where $mean_s$ is the summary mean obtained by the formula;

$$mean_s = \Sigma(w_i . mean_i)/ \Sigma(w_i) \qquad (eqn\ 3.27)$$

95% confidence interval(C.I),

$$95\% = mean_s \pm (1.96 . \sqrt{variance_s}) \qquad (eqn\ 3.28)$$

$$variance_s = 1/ \Sigma(w_i) \qquad (eqn\ 3.29)$$

iv. *Strength and limitations*

The methods employed above use standard deviation as a measure of outcome but are not accepted by all statisticians. Greenland, Schlesselman, and Criqui (1987) gave examples where studies with identical results can spuriously appear to yield different results when the effect measures are converted to units of standard deviation. The argument here is that when instruments and scales are different and unless the effect can be converted to a common metric, meta-analysis is impossible. Accordingly, when there is no reason to convert the measures of effect to unit of standard deviations, natural units should be used.

b. Estimating trends

Estimating trends between the risk of expose and unexpose groups towards certain condition is critical in the assessment of the causality of the association. Greenland (1987) describes a method to estimate the summary measure of risk or trend using the slope from regression equation. The method used ordinary weighted least square regression to estimate *b*(beta), the slope of the trend of disease. The formulas to estimate the summary measure of slope, a statistic to test homogeneity, and 95% confidence interval are as follows;

Step.1 Summary measure of slope

$$Slopes = \Sigma(w_i . slope_i)/ \Sigma(w_i) \qquad (eqn\ 3.30)$$

$$Weight(w_i) = 1/ variance_i \qquad (eqn\ 3.31)$$

where $slope_i$ and $variance_i$ is the slope and variance of the ith study [Greenland and Longnecker 1992 or Greenland 1992].

Step.2 Test of homogeneity

$$Q = \Sigma(w_i . (slope_s - slope_i)^2) \qquad (eqn\ 3.32)$$

where Q is referred to the chi-square distribution with degrees of freedom equal to the number of studies minus 1.

Step.3 95% confidence interval

$$95\%\ C.I = slopes \pm (1.96 . \sqrt{variance_s}) \qquad (eqn\ 3.33)$$

$$variance_s = 1/\Sigma(w_i) \qquad (eqn\ 3.34)$$

where the weight(w_i) are estimated as above.

3.4 Conclusions

This chapter presents the rationale and some common pharmacokinetic model and statistical methods that can/are employed on population based studies. Approaches on how population-based study data and outcome measurements should be summarized were defined. The significance of employing these statistical methods on population based studies and how the final outcome can affect in optimising therapy was highlighted.

The statistical methods proposed can be beneficial especially in the field of evidence based medicine where the medicine assessment of pharmacoepidemiology and pharmacotherapy can improve treatment strategies. It can be concluded that these statistical methodologies can act as a scientific tool to summarize, appraise and communicate the results and implications of various unanswered questions.

Chapter 4

Ethnicity in established antiepileptic drugs: A review of its importance in optimizing antiepileptic drug dosing

4.1 Introduction

The population pharmacokinetics of established antiepileptic drugs(AED) such as phenytoin, carbamazepine, sodium valproate and phenobarbitone in man had been previously reviewed [Bertilsson and Tomson 1986, Gram and Bentsen 1985, Lester] and validated [Evans et al 1991,Thomson & Broody 1992, Davis et al 1994]. The use of population pharmacokinetics in optimizing drug dosing is specially important during initiation of therapy since these drugs have different pharmacological and pharmacokinetic properties. These drugs are also known to be most effective and safe at certain therapeutic concentrations [Brody and Dichter 1997].

Dose and serum concentration relationship of phenytoin is well-known to behave nonlinearly due to the saturation of hepatic metabolism or is commonly referred as saturable kinetics. The relationship between dose and serum concentration of carbamazepine, valproic acid and phenobarbitone has been studied but has yet to be established. Reports on carbamazepine [Eadie and Tyrer 1980, Dodson 1987], sodium valproate [Schapel et al 1980, Henriksen and Johannessen 1982, Turnbull et al 1983, Schobben et al 1975] and phenobarbitone [Eadie et al 1977, Painter and Pippenger 1978] are still inconclusive and these drugs are currently thought to behave non-linearly. The reasons behind the non-linear behaviour of these drugs is the many reports of the existence of high intraindividual and interindividual variation on the way patients respond to these antiepileptics [Welty et al 1983].

Pharmacologically, all four antiepileptic drugs are metabolised in the liver, moderate(phenobarbitone) to highly protein bound(phenytoin, carbamazepine, valproic acid) and only the free-drug fraction is in equilibrium with the brain [Chadwick 1994] and is therefore pharmacologically active. Valproic acid demonstrate inhibitory behaviour towards liver P450 microsomal enzyme while the rest are inducers [Patsalos and Duncan 1993]. Autoinduction is unique to carbamazepine [McNamara et al 1978] and the saturable kinetics of phenytoin

[Bochner et al 1972, Borofsky et al 1972, Mawer et al 1974, Richens and Dunlop 1975] in high dosage can both make therapy complicated.

In theory, pharmacokinetic-ethnic differences are associated with the liver cytochrome P-450's activity across species. These are the liver enzymes involved in the metabolic clearance of many drugs. Pharmacokinetic parameters such as clearance(CL) can be considered to be the product of organ blood flow and organ intrinsic clearance. For a drug cleared by the liver, the systemic clearance is;

$$CLs = \frac{QCL1}{CL1 + Q}$$

Where Q is the organ blood flow and CL1 the intrinsic clearance of the organ. For metabolically cleared drugs such as phenytoin, CL1 relates to the parameters *Vmax or Km* of the enzymes involved [Smith 1991].

The level at which the liver is involved clearly shows that factors such as age, weight, height, sex, and certain disease conditions can play a vital part when using population pharmacokinetic parameters during therapy. Weight has been found to be an important criterion in estimating the population pharmacokinetics of phenytoin [Houghton and Richens 1975], carbamazepine [Summers and Summers 1989], sodium valproate [Chiba et al 1985] and phenobarbitone [Yukawa et al 1992]. Wilmore 1995 reviewed and reported that phenytoin, carbamazepine and sodium valproate are affected by age. Several authors have reported that age is linearly related to dose/serum level ratio in carbamazepine [Yukawa et al 1992], phenobarbitone [Rossi et al 1979] and sodium valproate [Cloyd et al 1993]. Certain diseases of the liver and kidney have been found to affect the protein binding capacity and pharmacokinetics of all four antiepileptic drugs [Thomson and Brody 1992]. Changes in protein binding in pregnancy is however more pronounced with phenytoin than the other antiepileptic drugs[Brody 1990b].

Studies on estimating population pharmacokinetic variables of antiepileptic drugs used different methodologies and treatment patient groups. The traditional and well-known method used is the individualized approach but the population approach is becoming more prominent [Jelliffe et al 1998]. The most commonly employed treatment methods used either healthy or diseased patients and then categorised them

into patient groups of children, adult or both. However studies on patients with different racial or ethnic background are less common and the few that were published dealt mainly with phenytoin.

Studies on phenytoin among different ethnic groups had been reviewed by Edeki and Brase (1995). Although, the influence of ethnicity on the population pharmacokinetic of carbamazepine has been reported by Yoon et al 1996, the few studies on phenobarbitone [Botha et al 1995] or sodium valproate [Botha et al 1995] showed its effects are insignificant. To date, the observations on carbamazepine, phenobarbitone and sodium valproate are yet to be substantiated.

Ethnicity and genetic differences has been quoted in several phenytoin studies [Houghton and Richens 1975, Kromann et al 1981] as being one of possible contributing factors. These differences are referred to as polymorphism and their occurences among different ethnic groups can lead to interindividual variation in the drug pharmacokinetic and pharmacodynamic characteristics. The explanation given for genetically controlled ethnic differences can be described by the concept of acetylation polymorphism [Clark 1985] and is meant by the distribution of acetylation phenotypes in different population.

Pharmacogenetic and phenotype differences and polymorphism in metabolism and their relation to ethnicity have been reviewed or reported in phenytoin [Grasela et al 1983, Edeki and Brase 1995, Odani et al 1997]. Pharmacogenetic aspects of phenobarbitone and carbamazepine in humans were investigated by Vessel and Page (1969) and Eichelbaum et al (1985). Evidence towards these relationships was reported for phenytoin and phenobarbitone and not for carbamazepine.

Reports on these relationships were however confined to certain population groups(American, European and Japanese) and their results are currently being generalised almost world-wide. A direct implementation of these published pharmacokinetic profiles onto the local population may be inappropriate and can lead to treatment failure. These values should be best used as a guideline only until a proper quantitative analysis of the local population pharmacokinetics are available. Thus, this review was designed to highlight the extent at which these relationship were studied and the impact of ethnicity on phenytoin, carbamazepine, sodium valproate and phenobarbitone pharmacokinetics.

4.2 Phenytoin

Phenytoin or 5,5-diphenyl-2,4-imidazolidinedione has been used as an antiepileptic drug to control generalized and partial epilepsy for more than fifty years. It is inexpensive and used worldwide. The pharmacokinetic properties of phenytoin are characterised by the saturable metabolic pathway, dose dependent kinetics and the therapeutic range of serum concentrations [Arnold and Gerber, 1969; Lund et al 1974; Chang and Glazko, 1982].

The problems of phenytoin therapeutic as currently used therapeutically are associated with that of drug metabolism, nonlinear pharmacokinetics, intraindividual, interindividual and interethnic differences [Evans et al 1992]. The nonlinear dose and serum concentration relationship in phenytoin is explained by the saturation of hepatic metabolism where the maximum rate of metabolism occurs at concentrations within the therapeutic range(10 to 20ug/ml), where small incremental increases can lead to large increases to plasma concentration. The lower end of the therapeutic range is still not well defined [Hayes 1993] and many patients with generalised epilepsies can be controlled at lower concentration. The upper end is often associated with adverse drug reactions such as lethargy, ataxia and nystagmus although there are patients observing excellent therapeutic responses at concentrations above 20ug/ml.

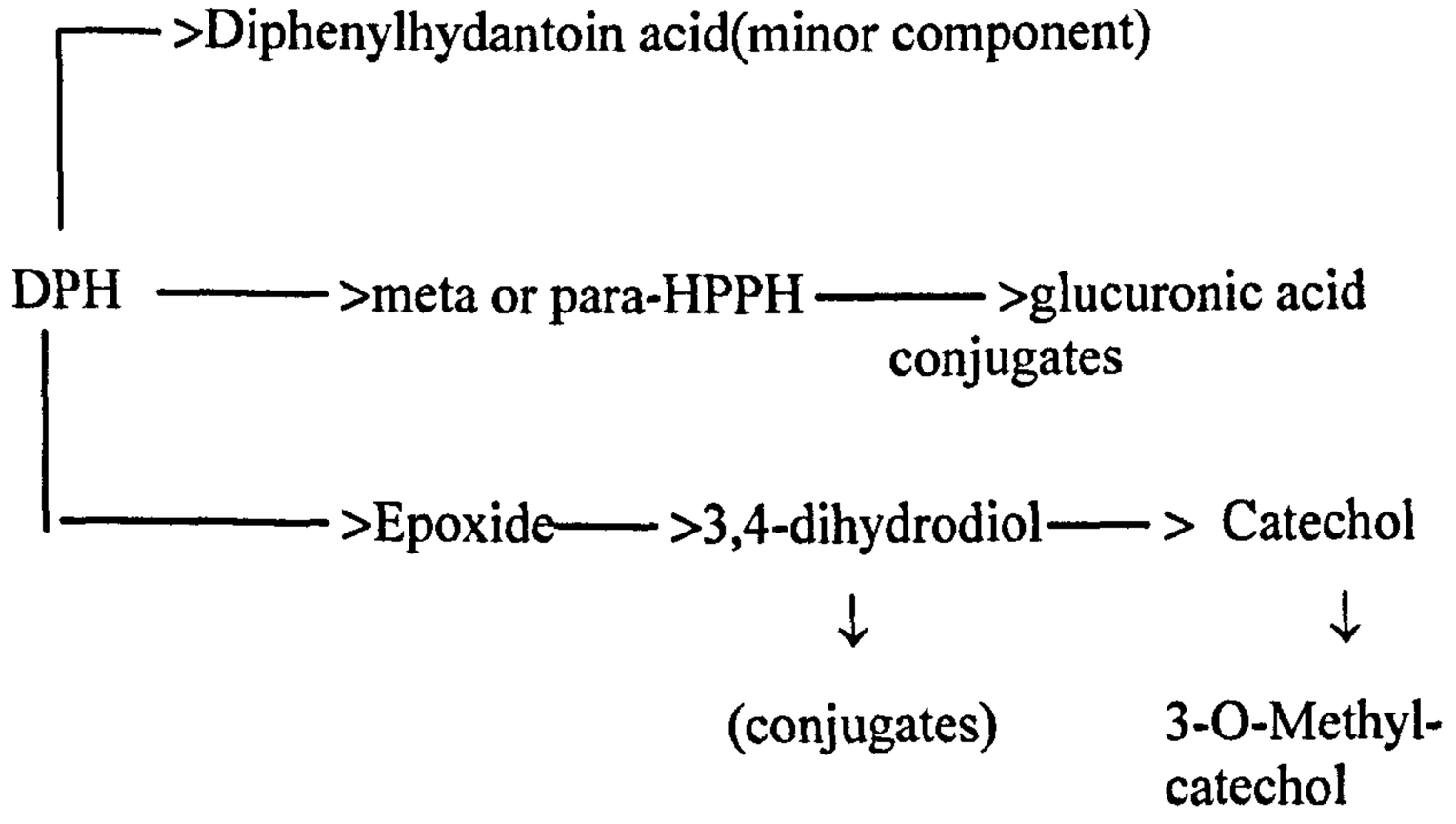
Population and individual based studies are currently investigating methods to reduce variations among individuals and across population. Studies on specific ethnic groups are still few in numbers but their potential importance may be crucial in the determination of phenytoin pharmacokinetics across population.

4.2.1 Variations in metabolism and pharmacokinetics

Glazko (1973) proposed the following pathways for phenytoin metabolism(figure 4-1). The main urinary metabolite is the glucuronic acid conjugate of the (S)-enantiomer of 5-(4-hydoxyphenyl)-5-phenylhydantoin(para-HPPH) [Fritz et al 1987]. The para hydroxylation is carried primarily by the family of cytochromes P450 called P4502C9 [Veronese et al 1991, Veronese et al 1993]. This enzyme also hydroxylates tolbutamide [Chen et al 1993] and warfarin [Rettie et al 1992, Kaminsky et al 1993]. Familial differences of reduce ability to hydroxylate phenytoin indicate a genetically link hypometabolism of phenytoin [Kutt et al 1964,Vasko et al 1980]. Individuals with this problem are observed to be having an apparent deficiency of

cytochrome P4502C9 and appears to be inherited as an autosomal recessive trait [Vermeij et al 1988]. These individuals are referred to as poor metabolisers and Inaba (1990) estimated the recessive trait to occur with a frequency of 1 in 500 in the Caucasian general population.

Figure 4.1:Proposed phenytoin metabolism and final metabolites



Studies on the relationship between ethnic groups and phenytoin pharmacokinetics are limited and difficult to compare. This is because these studies were distinctly different in both research methodology and outcomes measurement. These studies can however be broadly categorised into three main type; comparative, individualised and population studies.

4.2.2 Comparative studies

Table 4-1 highlighted some of the studies which reported observed differences between groups. The pharmacokinetic parameters reported were the rate of metabolism and disposition of phenytoin metabolite namely para-hydroxyphenytoin (p-HPPH), half-lives, clearance, Michaelis-Menten rate constant (*Km*), serum level/dose ratio and its steady-state concentration.

The relationship between ethnicity and p-HPPH is clearly demonstrated by Andoh et al (1980) where their results showed Black Ghanaians excreted the metabolite at a rate 3 to 4 times lower than Caucasians after a single 200mg dose. Although this suggests that Blacks would have a longer half-lives than Caucasians, results of Black population from Ghana and the United States showed otherwise.

Table 4-1: Comparative studies on phenytoin pharmacokinetics.

Reference	Population	Sample size	Dose / Route	Pharmaco-kinetic parameters	Conclusion
Dam et al 1974	Eskimos versus Caucasians	45:45	not stated oral	Total clearance	Eskimos > Caucasians
Dam et al 1977	Eskimos versus Caucasians	45:45	not stated oral	Steady-state concentration	Eskimos > Caucasians
Kromann et al 1981	Eskimos versus Caucasian	33:21	1.4-1.5mg/kg oral	Total clearance	Eskimos > Caucasians
Arnold & Gerber 1969	Blacks versus Caucasians	17:28	100mg tds X 3 days oral	half-lives($t_{1/2}$)	Blacks > Caucasians
Buchanan et al 1977	Blacks	12	18mg/kg oral	half-lives($t_{1/2}$)	Blacks < Caucasians
Andoh et al 1980	Blacks	8	single dose-200mg oral	Rate of excretion of p-HPPH	Blacks < Caucasians
Grasela et al 1983**	Japanese versus Caucasian	104: 218	Multi-dose retrospective	*Km, Vmax*	*Vmax*:is dependent on weight *Km*: Japanese < European
Peiris et al 1988	Caucasian versus Sri Lankans(Asians)	47:47	na prospective	serum level, relative serum concentration	Caucasian=Sri Lankan
Watanabe M et al 1997	Japanese: Normal vs slow metabolisers	13:5	Multi-dose prospective	*Km, Vmax*	*Vmax* : Slow metabolisers > normal metabolisers by 70% *Km* : No significant difference

The first published study on interethnic differences between Blacks and Caucasian was published about 29 years ago by Arnold and Gerber (1969). Their study reported the mean half-life of 26.5 hours of American Black volunteers was significantly higher than the mean of 18.5 hours in Caucasian. Buchanan 1977 later found that the half-life of adult Black paraplegic volunteers in South Africa(15.9 hours) to be similar to American Caucasian but gave no explanation was given for the observed difference. Edeki and Brase (1995) reported of an ongoing trial of Black American volunteers having a mean half-life(25.6 hours) similar to that reported by Arnold and Gerber (1969). The variation of results between American Blacks and African Blacks could be accredited to the probable presence of interethnic differences among the assumably homogenous Black population.

Dam et al (1974) and Kromann (1981) examined the difference in phenytoin clearance of the Eskimos and Caucasians population of Danish nationality. Both

studies showed that Eskimos total phenytoin and p-HPPH clearance were higher than Caucasian by fifty percent. The claim of interethnic differences were further strengthened by observations made by Dam et al (1977) which showed Eskimos steady-state serum concentration of phenytoin at a mean dose of 5.6mg/kg is significantly lower than Caucasian.

Sheiner et al (1982) and Grasela et al (1983) quantitatively compared phenytoin rate of metabolism(*Km*,mg/L) and the maximum elimination rate(*Vmax*,mg/day) between Japanese and Caucasian populations. Their results(mean,se) statistically showed that *Km* values of Japanese(3.8mg/L,0.65mg/L) are fifty-seven percent lower than Caucasian(5.7mg/L,0.61mg/L) patients(aged more than 15 years). For patients aged less than 15 years, their results showed that *Km* of the Japanese(2.2mg/L,0.18mg/L) population is about 67% of Caucasians(3.2mg/L,0.37mg/L). These indicate that the rate of metabolism in Japanese patients is almost half than that of Caucasians. Their results nevertheless showed that *Vmax* for the smaller body weight size Japanese population were statistically no different to that of Caucasian. These results again demonstrated the existence of interethnic differences between Japanese and Caucasian population

Other than interethnic differences, phenotype differences among population can lead to significant pharmacokinetic variation. Contradictory results among the black population from the continent of Africa and United States could be explained by their phenotype composition. Watanabe et al (1995) presented the importance of phenotype composition in estimating phenytoin population pharmacokinetic in a largely homogenous society of Japan. Their results showed that slow metabolisers had a significantly lower *Vmax* to that of normal volunteers. Similarly, Odani et al 1997 later found that slow metabolisers of genetic polymorphism of the CYP2C subfamily in the Japanese population had up to 14 percent lower *Vmax* that that of normal metabolisers. The rate of metabolism(*Km*) were however not affected by phenotype differences in Japanese population. This thus showed that other than ethnicity, phenotype differences can be the contributing factors for observed high intraindividual and interindividual variation on phenytoin pharmacokinetics.

4.3.3 Individual based studies

I. Studies on healthy volunteers

Hvidberg (1986) reviewed the pharmacokinetics of phenytoin on healthy Caucasian subjects. The trials consisted of reports from the United States, England, Denmark, and spanned the years of 1974 to 1981. To date, two studies of Black volunteers of Zimbabwean and American nationalities had been reported. A summary of these studies is presented in table 4.2.

Table 4.2: Phenytoin pharmacokinetics in healthy Caucasian and Black volunteers

Reference	Country	Sample size	Age(years) range or mean,sd	Dose/ Route of administration	Pharmacokinetic parameters
Arnold & Gerber 1969	Denmark -Caucasian and Blacks	28:17	21-54	100 mg X 3days oral	Half-lives
Buch Andreassen et al 1974	-Caucasian			100 mg(single), intravenous	Clearance
Perruca et al 1978	United States -Caucasian	3	26-38	92 mg(single) intravenous	Elimination constant(Ke), Half-live($t_{1/2}$), Volume of distribution(Vd), Clearance(CL)
Bach et al 1981	Denmark -Caucasian	28	young 28.8 , 8.3 elderly 83.5 , 7.1	100 mg(single) intravenous	Clearance
Kromann et al 1981	Denmark -Caucasian	21	23-62	100 mg(single) intravenous	Clearance
Hayes et al 1975	United Kingdom -Caucasian	41	20-86	oral/intravenous 60mg X 3 days	Clearance
Gugler et al 1976	United States -Caucasian	6	not stated	single 300mg oral single 300 intravenous 14 day X 300mg /day oral	Half-lives($t_{1/2}$), clearance
Nhachi et al 1992	Zimbabwean -Blacks	15	>22 years	200 single dose/oral/ prospective	Half-lives($t_{1/2}$)
Edeki & Brase 1995	United States -Blacks	58	adult	100 mg single oral dose	Half-lives($t_{1/2}$)

As indicated in table 4-2, studies were generally on adult volunteers given phenytoin either by the oral or intravenous route. These trials objectively investigated the half-life and clearance for both intravenous and oral preparation of phenytoin. Individuals selected consisted mostly of adults although those in the elderly age(more

than 65 years old) were included. Nevertheless, one trial did examine the influence of individuals in the elderly age group on phenytoin pharmacokinetics [Bach et al 1981]. This study compared the difference in clearance in young and elderly adults with the mean(SD) age of 28.8years(8.3years) and 83.5years(7.1years). Studies on the mixed age group patients by Dam et al (1977) consisted of patients in the range of 4 to 60 years. Summary of the final results reported by each study is in Table 4-3.

Table 4-3: Results of phenytoin pharmacokinetics in healthy Caucasian and Black volunteers

Reference	Country	Half-lives (hours) -mean,se	Elimination rate constant($hour^{-1}$) -mean,se	Volume of distribution (Liters/kilogram) -mean,se	Clearance range (ml/min) -mean,se
Arnold & Gerber 1969	United States -Caucasian	7-42 -18.5 , 1.15	-	-	-
Arnold & Gerber 1969	United States -Blacks	11.5-38.5 -26.5 , 1.94	-	-	-
Buch Andreassen et al 1974	Denmark -Caucasian	-	-	-	6.5 - 43.2
Perruca et al 1978	United States -Caucasian	8.3-38.1 -24.10 ,8.65	0.04 ± 0.04	41.53 ± 9.08	37.06 ± 26.45
Bach et al 1981	Denmark -Caucasian	adults 26.5 , 2.36 elderly 29.5 , 2.42	-	adult(Liters) 40.9 ± 8.5 elderly(Liters) 44.4 ±18.1	adults 48.1± 4.44 elderly 50.5 ±9.86
Kromann et al 1981	Denmark -Caucasian	-	-	-	18.3 - 81.7
Hayes et al 1975	United Kingdom -Caucasian	-	-	-	adult:- 30.5 ± 4.5 Elderly:- 49.3 ± 6.5
Gugker et al 1976	United States -Caucasian	-	-	-	25 ± 2.5
Nhachi et al 1992	Zimbabwean -Blacks	36.7-40.5 -40.2 ,7.36	-	-	-
Edeki & Brase 1995	United States -Blacks	11.5 - 54 -25.6 , 2.39	-	-	-

Studies reporting on the half-lives of phenytoin after single oral doses showed a clear distinction between adult Caucasian and Black population. Generally the half-lives(95% confidence interval) in the black population ranged between 22.70-33.30 hours [Arnold & Gerber 1969], 20.92-30.28 hours [Edeki & Brase 1995] and 25.85-

54.62 hours [Nhachi et al 1992] were significantly higher than that of Caucasians [Arnold & Gerber 1969:16.25-20.75 hours]. However, the mean half-lives obtained from single dose intravenous studies by Perruca et al (1981) (7.15-41.05) and Bach et al (1981) (21.87-31.13) showed no such difference. This observation can possibly be explain by the different mode of administration and the existence of genetic polymorphism among the Caucasian volunteers [Horsmans et al 1997].

ii. Studies on epileptic volunteers

Table 4-4 highlighted eleven individual-based trials in Caucasian, Japanese, Chinese, Blacks, Arabs, Danish Greenland Eskimos and Malaysian epileptic patients. The pharmacokinetic parameters investigated were the Michealis-Menten constant(*Vmax, Km*), estimated by employing the modified linearised Michealis-Menten equation, half-life($t_{1/2}$) and dose/serum level ratios.

Studies on the metabolic rate constant(*Km*) on paediatric and adult Caucasians showed that there are no significant differences between the population of Germany, England and United States. The reported *Km* values(95% confidence interval,mg/L) for the adult population of 5.48-6.54mg/L, 4.89-13.91mg/L and 5.4-5.8mg/L were homogenous although the values from England showed a wider coefficient of variation. *Km* values of 5.06-9.93mg/L and 6.08-6.92mg/L for the paediatric population nevertheless showed that inter-individual differences was more pronounced in the English Caucasian population.

Results from the Asian community also showed similarities between Japanese, Saudis and Malaysian population. Studies between Saudi(4.45-9.03mg/L) and Japanese(2.88-4.52mg/L) paediatric population showed that the *Km* values of the former were higher than that of Japanese but the difference was not statistically significant.

Comparing mixed population *Km* values for Malaysian(4.03-9.40mg/L), Saudis(5.18-8.32mg/L) and Caucasians of German origin(5.48-6.54mg/L) again showed the absent of interethnic differences between them. Significant difference was however present between Japanese [Chiba et al 1980] and Caucasian paediatric populations. *Km* values for the Caucasian range between 5-9mg/L was lower than that

observed in the Japanese population(2.88-4.52mg/L). This thus showed that evidence of interethnic differences is apparent and needs investigation.

Table 4.4: Phenytoin individualised-based pharmacokinetics in epileptics of various ethnic groups

Reference	Population (country)	Sample size	Age range -mean, sd(yrs)	Dose / route / study type	Pharmaco-kinetic parameters	Overall results (mean, se)
Rambek et al 1979	Caucasian (Germany)	178	6-72 years	Multi-dose / oral/prospective	K_m, V_{max}	K_m: 6.01 , 0.27mg/L) V_{max}: 355 , 7.94(mg/day)
Cranford et al 1977	Caucasian (Unites States)	139	17-94 mean-52	continuos infusion (mean dose-18 mg/kg)/prospective	clearance, volume of distribution	CL: 0.0157, 0.0011 liters/hr.kg V_d:0.78 , 0.0.01L/kg
Dam et al 1977	Caucasian (Denmark)	45	4-60 mean: 21.3 years	not stated/ oral/prospective	Clearance	CL:17 , 7.90 ml/min
Dam et al 1977	Eskimos (Denmark)	45	5-59 mean: 20.7 years	not stated/oral/ prospective	Clearance	CL: 66.54 , 25.03 ml/min
Chiba et al 1980	Japanese (Japan)	104	<16 years	Multi-dose/oral/ prospective	K_m, V_{max}	K_m: 3.7, 0.42(mg/L) V_{max}: not shown but stated to decrease with age
Blain et al 1981	Caucasian (England)	40	children: <8 years	Multi-dose/oral/ prospective	K_m, V_{max}	Children: K_m-7.5 , 1.24 (mg/L) V_{max}- 20.4 , 2.07 mg/kg.day or 238, 125.5 mg/day
			>18 years			Adult- K_m-9.4 ,2. 3 (mg/L) V_{max}- 8.7 , 0.71 mg/kg.day or 542.2, 37 mg/day
Baur et al 1983	Caucasian (United States)	92	>20 years	Multi-dose/oral / prospective	K_m, V_{max}	V_{max}: ~2 -11 (mg/kg/day) K_m: 5.4 - 5.8 (mg/L)
Baur et al 1983	Caucasian (United States)	155	<16 years	multi-dose /oral/prospective	K_m, V_{max}	K_m: not increase with age -6.26,0.09(mg/L) V_{max}: increase with age -10.22, 2.27 (mg/kg.day)
El-Sayed et al 1989	Saudis (S. Arabia)	17	mixed age children <18 years -5 adult -12	Multi-dose/oral /prospective	K_m, V_{max}	Children: K_m- 6.74 , 1.17 (mg/L) V_{max}-10.20 , 1.01 (mg/kg.day) or 234, 54.85mg/day Adult: K_m-6.75 , 1.08 (mg/L) V_{max}-6.87, 1.55 (mg/kg.day) or

						433.75,19.72mg/day V_{max} is dependent to age and weight
Lai 1985	Chinese (Taiwan)	70	mixed age	Multi-dose/oral/ prospective	Steady-state serum concentration	Dose and not age nor sex affected dose/serum level relationship
Lee et al 1989	Chinese/ Malay/ Indian (Singapore)	330	>15 years	multi-dose /oral/prospective	steady-state/dose ratio	Indian > Chinese and Malay
Ismail & Fatah 1990	Malay/ Chinese (Malaysia)	15	mixed age	Multi-dose/oral/ prospective	K_m, V_{max}	K_m: 6.72 , 1.37(mg/L) V_{max}: 8.45 , 0.36 (mg/kg.day) or 406 39, 31.66mg/day

Vmax among mixed populations of Caucasian origin by Rambek et al 1979(339.43-370.56mg/day), Saudis(317.08-432.92mg/day) and Malaysian(344.34-468.44mg/day) patients showed no significant difference. However, the results from the Malaysian population might not portrayed the true picture since the values were from a mix of Malay and Chinese patients. This fact was clearly showed by Lee et al 1989 where the serum concentration/dose ratio of Chinese was higher than that of Malay patients and could probably affect the overall mean(*Vmax*) of the Malaysian population.

Reported *Vmax* values for Caucasian paediatric population by Blain et al (1981) (16.34-24.46mg/kg.day) and Baur et al (1983) (5.77-14.66mg/kg.day) were found to differ significantly. However, the possibility that inter-ethnic differences existed between American and English population cannot be confirmed due to different patient study age groups. Studies on similar age group paediatric patients by El-Sayed et al (1989) and Baur et al (1983) showed no significant difference was observed between Saudis(8.22-12.18mg/kg.day) and Caucasians(5.77-14.66mgkg.day).

4.3.4 Population-based studies

Altogether, ten trials published phenytoin pharmacokinetic trials on specific ethnic populations. The trials covered eleven different nationalities from four different continents namely North America, Europe, Africa and Asia. Their diverse ethnic

background can be classified into Caucasian, Japanese, Chinese, Blacks, Arabs, and a mix of Malay, Chinese and Indian from the multi-racial country of Malaysia.

Table 4.5 summarises ten population-based studies of phenytoin which mentioned the ethnicity of the studied epileptic groups. These studies employed the non-linear mixed effect model by using specialised computer software(OPT and NONMEM) in estimating the population pharmacokinetics of each population groups.

Table 4.5: Population-based studies of epileptics of various ethnic groups using computer software of non-linear mixed effects models(OPT** and NONMEM*)

Reference	Population (country)/	Sample size	Age (yrs)	Dose / route /study type	Pharmaco-kinetic parameters	Overall results (mean,se)
Voseh et al 1981*	Caucasian (Switzerland) -epileptics	32	>22 years	Multi-dose, retrospective	K_m, V_{max}	K_m: 7.22 , 0.25 mg/L V_{max} : 4.44 , 0.40 mg/kg.day
Grasela et al 1983*	Caucasian (Germany) -epileptics	178	mixed age	Multi-dose retrospective	K_m, V_{max}	K_m:5.7 , 0.21 V_{max} :355 , 5.32 mg/day
Grasela et al 1983*	Japanese (Japan) -epileptics	104	<16	Multi-dose retrospective	K_m, V_{max}	K_m:2.2 , 0.12mg/L V_{max}:208 ,2.45 mg/day
Grasela et al 1983*	Caucasian (England) -epileptics	40	<8	Multi-dose retrospective	K_m, V_{max}	K_m:3.2 , 0.25mg/L V_{max}:138 ,4.36 mg/day
Miller et al 1987*	Blacks (S.Africa) -epileptics	37	mixed - age	Multi-dose retrospective	K_m, V_{max}	K_m:3.43 , 0.27mg/L V_{max}:6.5 , 0.28 mg/kg.day
Chan et al 1990**	Chinese (Singapore) -epileptics	121	mixed age	Multi-dose retrospective	K_m,	K_m: 2.31 , 0.04mg/L no difference between children and adult
Yukawa et al 1990*	Japanese (Japan) -epileptics	220	mixed age	Multi-dose, retrospective	K_m, V_{max}	K_m(60kg individual): 3.67 , 0.28mg/L V_{max}(60kg individual): 369 , 9.82mg/day or 6.15 , 0.16mg/kg.day
Ismail & Fatah 1994**	Malay/Chinese/ Indian (Malaysia) -epileptics	59	mixed age	Multi-dose retrospective	K_m, V_{max}	K_m:3.66 , 0.77 mg/L V_{max}: 7.29 , 1.23 mg/kg.day
Rui et al 1995*	Chinese (China) -epileptics	161	mixed age	multi-dose retrospective	K_m, V_{max}	K_m: 6.21, 0.22 mg/L V_{max}: 439, 5.37 mg/day or 7.32 , 0.09 mg/kg.day (60kg individual)
Odani et al 1996*	Japanese (Japan) -epileptics	116	Mixe d age	Multi-dose retrospective	K_m, V_{max}	K_m:9.19,1.08mg/L V_{max}:9.80 ,0.51 mg/kg.day

i. ***Km***

The reported *Km* values(95% confidence interval) in the mixed-age group of the Caucasian(5.29-6.12mg/L) and China-Chinese(5.78-6.64mg/L) was definitely higher than those of Japanese(3.12-4.22mg/L)), Black South African(2.90-3.96mg/L), Singapore-Chinese(2.23-2.38mg/L), and Malaysian mixed race(2.15-5.17mg/L) patients. These differences showed that interethnic differences could possibly exist. Similarly values for the paediatric Japanese(1.96-2.43mg/L) and Caucasian(2.71-3.69mg/L) population showed further proof for interethnic difference.

As for the adult population, the values for Caucasian(6.73-7.71mg/L) is at least 2 times higher than that of Chinese patients(2.23-2.38mg/L) obtained by Chan et al 1990. Although Chan et al (1990) reported no significant difference for *Km* between children and adult, a clear distinction was observed in Caucasians. Results by Voseh et al (1981) for adults(6.73-7.71mg/L) and Grasela et al (1983) for children(2.71-3.69mg/L) showed age can play an important factor among Caucasians.

ii. ***Vmax* (Maximum elimination rate)**

Population values of *Vmax*(mg/day) from mixed age group from mainland Chinese(428.87-449.52mg/day) was definitely higher than Caucasian(344.57-365.43mg/day) or Japanese(349.75-388.25mg/day). *Vmax* values in mg/kg.day similarly showed that there was no significant differences existed between mainland Chinese(7.14-7.50mg/kg.day), Black South African(5.95-7.05mg/kg.day) and multiethnic Malaysian population(4.88-9.70mg/kg.day).

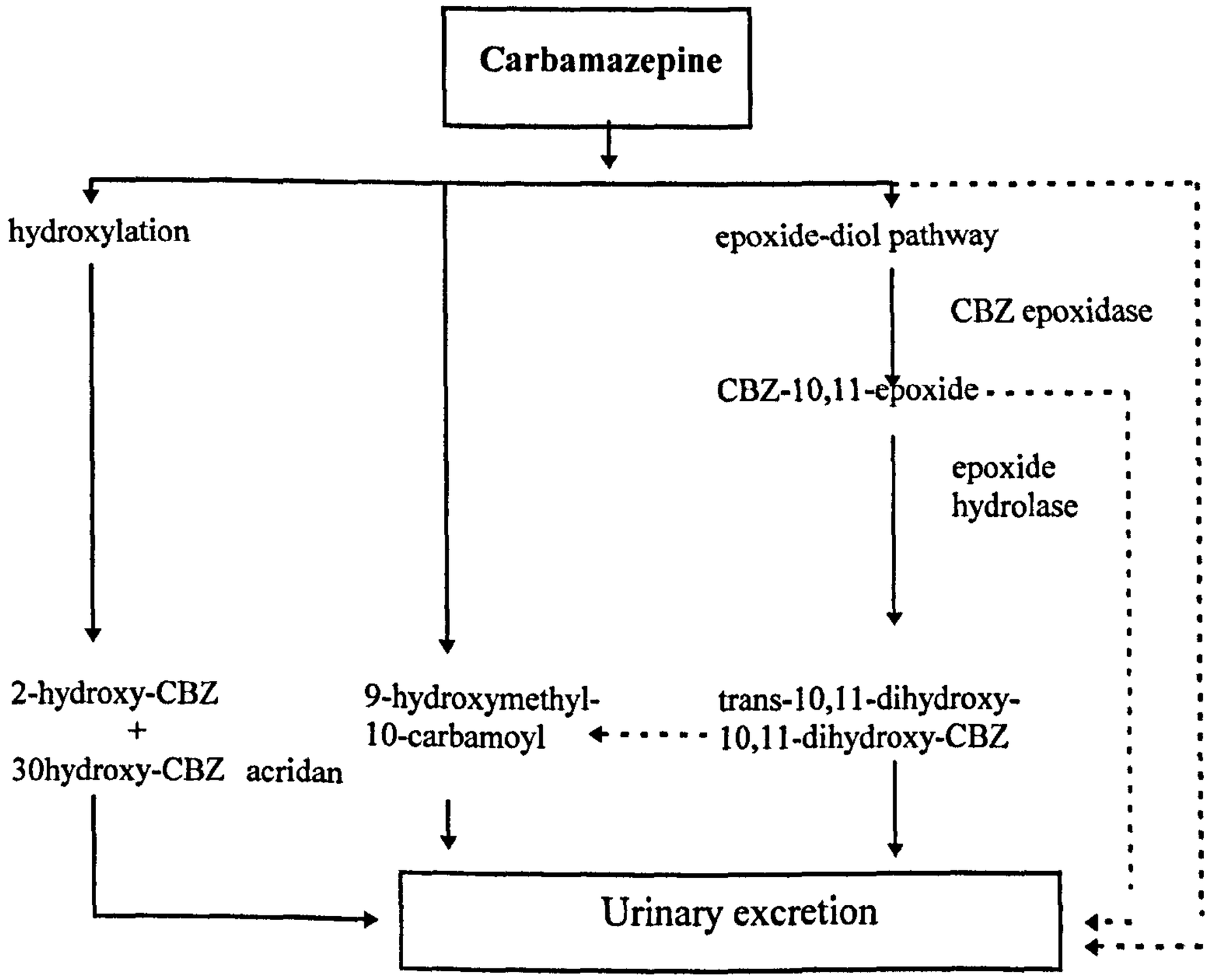
Vmax between Japanese and Caucasian paediatric population nevertheless displayed evidence for interethnic differences. Figures obtained for the Japanese population was about 33 percent lower than their Caucasian counterpart. The difference between adult and paediatric Caucasian could not be studied since the units used were incomparable.

4.4 Carbamazepine

Carbamazepine(CBZ) is first discovered in 1952 and currently considered as the first line treatment of both partial and generalized epilepsy [Wilder 1995]. Metabolism of carbamazepine has been studied both in-vivo and in-vitro [Eichelbaum

et al 1984, 1985, Lertratanangkoon & Horning 1982, Tybring et al 1981]. The most important pathway is that describe by Eichelbaum (1985) where the active metabolite namely called carbamazepine-10,11-epoxide is formed.(figure 4-2).

Figure 4-2: Major pathways of metabolism of carbamazepine in man(Eichelbaum et al 1985).



CBZ pharmacokinetics has been extensively reviewed and its autoinduction properties has been well documented in Caucasians [Eichelbaum et al 1985, Bertilsson et al 1986, Mikati et al 1989, Rapeport et al 1983]. The mechanism of autoinduction is reported by Rapeport et al (1983) and to be attributed to the activation of hepatic microsomal enzyme.

The role of genetic factors in the interindividual variability in carbamazepine is little known although interethnic differences in drug metabolism among different population is often cited [Rodorfer et al 1985, Kalow et al 1984, Kalow 1982] and have been reported for other drugs. Eichelbaum et al (1985) investigated the possibility of this pharmacogenetic link upon eight Swedish volunteers who were then

subjected to seven metabolic reaction test that are associated for interindividual difference. These test which included debrisoquine 4-hydoxylation [Mahgoub et al 1977], sparteine oxidation [Eichelbaum et al 1979], and antipyrine [Blain et al 1982, Vesell and Page 1968], examined carbamazepine clearance via its epoxide metabolite and found no significant relationship in any of the tests. They then concluded that the metabolism and clearance of carbamazepine were dependent on types of therapy and not to its pharmacogenetic aspects.

Marley et al (1993) however found that the time course for anticonvulsant effect of carbamazepine on cocaine-induced seizures in rats depends on its genotype. The levels of carbamazepine and its active epoxide metabolite(CBZ-10,11-epoxide) in plasma and brain were dependent on strain-type which clearly indicates that there were genetic differences in its time course. These results showed the possible existence of interethnic differences in humans which has yet to be established.

4.4.1 Individual-based studies

Table 4-6 presented five published studies of healthy adult volunteers and a single study on epileptics which examined various carbamazepine pharmacokinetic parameters in single and chronic therapy. The most common parameter studied was the half-life of carbamazepine although several studies did measure parameters such as absorption rate constant(k_a),elimination rate constant(k_e) and volume of distribution(V_d).

Results on the half-life of carbamazepine was obviously not affected by dose [Levy et al 1974] and half-lifes among healthy Caucasians was distinctly similar(Table 4.6a). The reported half-lifes(95%,confidence interval,hours) for Caucasians in single dose studies on healthy volunteers were 27.15-38.05 hours [Levy et al 1974], 21.49-30.51 hours [Tomson et al 1983], 29.15-40.25 hours [Dalton et al 1985a], and 27.77-36.63 hours [Dalton et al 1985b] respectively. Results reported by Eichelbaum et al (1979) on epileptics patients although similar showed a wider range than in healthy volunteers(20.61-50.59 hours).

Interethnic differences of half-lives between Koreans(44.13-52.44 hours) and Chinese(43.10-57.10 hours) were similarly small. No evidence of interethnic differences were also observed between healthy Caucasians from the United

States(27.15-38.05 hours, 29.15-40.25 hours, 27.77-36.63 hours) and Sweden(20.61-50.59 hours). However, the possibility of differences between Caucasians and Asians of Chinese and Korean nationalities might be present.

Table 4-6: Carbamazepine pharmacokinetics in various ethnic groups

Reference	Ethnic(Country)/ Patient-type	Sample size	Age(years, mean, sd)/ Ratio male:Female/ Weight(mean,sd)	Dose/ Route of administration	Pharmacokinetic parameters
Levy et al 1974	Caucasian (United States) -healthy volunteers	6	-25 ± 3.8 yrs -3 : 3 -69.3 ± 18.8 kg	single dose-6mg/kg p.o	half-life($t_{1/2}$)
Tomson et al 1983	Caucasian (Sweden) -healthy volunteers	4	-35.5 ± 10.1 yrs -2 : 2 -not stated	single dose-200 mg p.o	Elimination rate constant(Ke), Half-live($t_{1/2}$), Volume of distribution(Vd), Clearance(CL)
Hundt et al 1983	Blacks versus Caucasians (United States)	451: 2225	na	multiple dose	mean serum concentration, CBZE/CBZ and CBZH/CBZ ratios
Eichelbaum et al 1985	Caucasian (Germany) -epileptic	6	31.5 ± 3.7yrs -3 : 2 -66.6± 16.06kg	single-100 or 200 mg p.o + other AED's	half-life($t_{1/2}$), Vd
Dalton et al 1985	Caucasian (United States) -healthy volunteers	8	24 - 35yrs -7:0 -81± 9.6kg	single- + cimetidine (600mg)	half-life($t_{1/2}$)
Dalton et al 1985	Caucasian (United States) -healthy volunteers	8	23 - 36yrs -7:0 -81.0 ± 9.6kg	single-600mg + rantidine 300mg/day	half-life($t_{1/2}$)
Peiris et al 1988	Caucasian versus Sri Lankan	47:47	10-72yrs wt-na	multiple dose	serum level, relative serum concentration(1/CL)
Lin et al 1991	Chinese(Taiwan) -healthy volunteers	12	-23.4 ± 3.1yrs -7: 0 -67.7 ± 11.2kg	a. Single dose(200mg) + 600mg phenytoin(p.o) b. Multiple dose 200mg tds X 7days, last dose + 600mg phenytoin(p.o)	Vd, Ka, Ke and $t_{1/2}$
Yoon et al 1977	Koreans(South Korea) -healthy volunteers	139	22.6 ± 1.3 -67.5 ± 5.7 kg	a. Single dose-400mg b. Multiple dose 200/day X 2 weeks	Vd, Ka, Ke and $t_{1/2}$

Volume of distribution(*Vd*) of healthy Caucasian(0.64-1.11 L/kg) was lower than that reported by Korean(1.22-1.62 L/kg) and Taiwan-Chinese(1.01-1.29 L/kg). However, these differences were not significant but showed that Caucasian had higher inter-individual variation. Calculated V_d of Korean and Taiwanese Chinese

similarly showed the existence of differences in inter-individual variation between them.

Table 4-6a : Summarised results of carbamazepine studies in various ethnic groups.

Reference	Ethnic groups	Half-lifes (t1/2-hours) Mean,se	Elimination rate onstant(ke-L/hr) Mean,se	Volume of distribution (Vd-L/kg) Mean,se	Serum level (mg/L) Mean,se	Clearance (ml/kg.hr) Mean,se
Levy et al 1974	Caucasian (United States) -healthy volunteers	single dose(3mg/kg) -32.60 , 2.78 single dose(6mg/kg) -30.60 , 1.88 single dose(9mg/kg) -32.60 , 5.02	na	na	na	na
Hundt et al 1983	Blacks vs Caucasians (United States)	na	na	na	Blacks> Caucasian	na
Tomson et al 1983	Caucasian (Sweden) -healthy volunteers	single dose(200mg) 26.00 , 2.30	na	0.88 , 0.12	na	23.40 ,2.30
Eichelbaum et al 1975	Caucasian (Sweden) -disease	single dose(100mg) -35.60, 7.65 multiple dose(200mg) -20.90 ,2.50 combine -8.20 ,1.17	na	single dose -1.20, 0.22 multiple dose -not stated combine -1.12, 0.09	na	single dose -30.4, 9.90 (ml/min) multiple dose -not stated combine -114, 9.44 (ml/min)
Dalton et al 1985a	Caucasian (United States) -healthy volunteers	single dose(600mg) 34.7 ,2.83	na	na	na	na
Dalton et al 1985b	Caucasian (United States) -healthy volunteers	single dose(600mg) -32.20, 2.26	na	na	na	na
Peiris et al 1988	Holland (Caucasian) vs Sri Lankan (Indians)	na	na	na	Caucasian -7.54,2.0 S.Lankan -7.45,3.22	RSC(mean,se) Caucasian -0.66 , 0.24 Sri Lankan -0.67 , 0.52
Lin et al 1991	Chinese(Taiwan) -healthy volunteers	single dose(400mg) 50.1 ,3.57 multiple dose(600mg) -26.10, 1.48	single dose -0.015 , 0.001 multiple dose -0.027, 0.002	single dose 1.15 , 0.07 multiple dose -1.64, 0.24	na	Sngle dose -16.40 , 1.17 Multiple dose -45.30 , 8.27
Yoon et al 1997	Koreans(South Korea) -healthy volunteers	Single dose(400mg) -48.29,2.12 Multiple dose(200mg) -26.97,1.41	Single dose -0.016,0.001 Multiple dose -0.027,0.001	Single dose -1.42 , 0.10 Multiple dose -1.36 , 0.08	na	Single dose -21.14,1.21 Multiple dose -35.08,1.53

Hundt et al (1983) reported mean total carbamazepine, CBZ-epoxide, and CBZ-dihydroxy level of Black patients($n = 451$) visiting the outpatients clinic and found them to be significantly lower($p<0.0005$) than Caucasians($n = 2225$). They also mentioned the difference between blacks and Caucasians could be due to Blacks having lower therapeutic window and elimination rate constant and the possibility that comedication tended to have greater impact on them. However, the prevalence of

higher incidences of noncompliance would raised the question of adequacy among Blacks. Peiris et al (1988) however compared dose and serum level relationship of Dutch and Sri Lankan patients and found no significant differences between them. They further noted that the wider variabilities in dose-serum concentration in Sri Lankan patients might possibly be due to noncompliance.

Carbamazepine clearance although known to be dose dependent [Kudriakova et al 1992, Summers & Summers 1989], was observed to be higher in Caucasians than Korean or Taiwanese Chinese but no difference was observed between the latter two groups. This finding was obvious since clearances(95% confidence interval) for Caucasian after a single dose of 200mg(18.89-27.91 ml/kg.hr) were markedly higher than the 400 or 600 single dose taken by Taiwan-Chinese(14.11-18.69mg/kg.hr) or Korean(18.77-23.51 mg/kg.hr) volunteers. The determination of the elimination rate constant(k_e), which is one of the two variables that has a direct relationship with clearance, again showed that no such relationship occurred between Chinese and Korean volunteers.

4.4.2 Population-based studies on carbamazepine in epileptic patients from various ethnic groups.

Studies on ethnic-based population pharmacokinetics of carbamazepine are again very few and until today it is not surprising that the relationship between dose and serum levels has yet to be concluded(Table 4-7). These studies tried to establish the influence of ethnicity to this undetermine relationship [Cereghino et al 1973, Summer & Summers 1989, Ismail & Rahman 1993] as well as investigating the relationship between parameters such as serum level-dose ratio [Yukawa et al 1985] to variables such as age, weight etc. To date, the most common population pharmacokinetic parameter examined by researchers was clearance [Yukawa 1995, Ismail & Rahman 1993, Summers & Summers 1989] and this determinant are used to compare differences among ethnic groups.

The relationship between serum level and dose was observed to be linear in adult Caucasians [Cereghino et al 1973, Yukawa et al 1985], and curvilinear in paediatric Black South African patients [Summers & Summers 1989]. Ismail and Rahman (1993) however found no clear relationship between serum level and dose in Malaysian patients.

Table 4-7: Carbamazepine population pharmacokinetics of various ethnic groups

Reference	Ethnic(Country)/ Patient-type	Sample size	Age(years) Mean,sd/ range	Dose/ Route of administration	Pharmacokinetic parameters/Methods of analysis
Yukawa 1995	Japanese (Japan) -epileptics	317	14.9 , 9.2	Multi-dose -retrospective	-CL -Non-linear mixed effect model(NONMEM) using multiple peak approach
Ismail & Rahman 1993	Malaysian (Malaysia) -epileptics	20	11-37	Multi-dose -prospectve	-Elimination constant(Ke), Absorption rate constant(Ka), Volume of distribution(Vd), $t_{1/2}$ Clearance(CL), serum level and dose relationship -Bayesian(OPT)
Yukawa et al 1985	Japanese (Japan) -epileptics	370	<18	Multi-dose -retrospective	-Serum level/Dose ratio, serum level and dose relationship -linear regression
Summers & Summers 1989	Black African (South Africa) -epileptics	262	<18	Multi-dose -retrospective	-CL, and serum level and dose relationship. -Linear regression
Cereghino et al 1973	Caucasian (United States) -epileptics	8	21-48	Multi-dose -prospective	-Serum level and dose relationship -Linear regression

Population clearances estimated for Japanese and Malaysian were found to be significantly different. Their clearances range(mean,95% confidence level) were 68.10,66.47-69.72 ml/kg.hr and 60,55.53-64.47 mg/kg.hr showed that Malaysian patients values was about 10 percent lower than that of Japanese. Summers and Summers (1989) further reports that clearances in black paediatric patients decrease with increasing body mass, but increase with dose. Their correlation relationship for clearance, body mass and dose had an r value of 0.66 which they commented could be affected by other factors such as diet or genetic makeup. Delgrado Iribarnegaray (1997) also reported similar observations and mentioned that the nonlinearity relationship between clearance and age would mean that older children would have lower clearance with respect to total body weight.

4.4 Sodium Valproate

Valproic acid is a branched-chain fatty acid and is available in its parent compound, sodium salt, its amide derivative, and a combination of the parent compound and its sodium salt. It is eliminated mostly by metabolism and recovered less than 5% in the urine. Studies in mouse showed two active metabolites of valproic acid are the 2-En-valproic acid and 4-En-valproic acid [Loscher & Nau 1985].

The pharmacokinetics of sodium valproate have been reviewed by various authors [Simon et al 1975, Pinder et al 1977, Lewis 1978, Bruni et al 1979, Koch Weser et al 1980, Gram et al 1985, Davis et al 1994] and its pharmacokinetic properties are well established. Its serum level and dose relationship at steady-state has been described as linear or dose dependent clearance [Hall et al 1985, Nutt & Kupferberg 1979], curvilinear [Gram et al 1979, May & Rambek 1985, Turnbull et al 1983] and the serum level dose ratios decreases with dose [Tisdale et al 1992]. The reasons for the contradictory observations were explained by the drug saturated protein binding at high dosages [Davis et al 1994] resulting in increased clearance.

Its pharmacokinetic properties in a variety of formulation [Chun et al 1980, Albright et al 1984, Barre and Berger 1989, Bailer et al 1985] have been studied in both paediatric and adult diseased patients and healthy volunteers. Predominantly pharmacokinetic parameters were significantly affected in patients in the paediatric and elderly age. Parameters such as $t_{1/2}$, *Vd* and percentage of unbound were found to increase more in neonates than those in infants or children [Morselli et al 1980] while the older people has 2 fold prolongation of $t_{1/2}$ and a higher *Vd* [Bryson et al 1983].

The effects of pregnancy, renal disease [Brewster & Muir 1980, Robson et al 1980, Gugler & Mueller 1978], hepatic dysfunction [Klotz et al 1978] and disease such as diabetes mellitus [Gatti et al 1987] had also been assessed. All these diseases increased the unbound portion of the drug and this increased the risk of hepatotoxicity. Hence dosage adjustment based on the total plasma concentration should be made cautiously.

All the above results have yet to ascertain interpatient variability due to ethnic differences. Comparison among ethnic groups were impossible since rarely was this factor mentioned. Until recently, a few studies did investigate the role and importance of intraindividual and interindividual variation [Chiba et al 1985, Botha et al 1995, Tisdale et al 1992] among population and their findings are discussed below.

4.3.1 Ethnic-based studies

Two studies of the paediatric population [Chiba et al 1985, Botha et al] using the individualised and population approach mentioned specific population or ethnic race in their trials(Table 4-8). Chiba et al 1985 compared Japanese children on

monotherapy and polytherapy and found that mean clearances of patients on polytherapy(0.023L/kg.hr) were significantly higher($p<0.001$) than monotherapy(0.013L/kg.hr) patients. These differences were not observed by Botha et al 1995b who studied a mix of Black and Indian paediatric South African patients population. The clearances values(95% confidences, L/kg.hr) were 0.21(0.018,0.024) and 0.028(0.023,0.033) for patients on monotherapy and polytherapy respectively showed no such differences. This result was similarly obtained by Cloyd et al (1993) for both monotherapy(0.005-0.024L/kg.hr) and polytherapy(0.016-0.085L/kg.hr) paediatric patients. This study however had its drawback of not mentioning patients ethnic composition thus preventing a direct interethnic comparison among studies.

Table 4-8: Sodium valproate population pharmacokinetics of various ethnic groups

Reference	Ethnic(Country)/ Patient-type	Sample size	Age(years)/ weight(kg) Mean,sd	Dose/ Route of administration	Pharmacokinetic parameters/Methods of analysis
Chiba et al 1985	Japanese (Japan) -epileptics	37	monotherapy age-9.4 , 2.9 polytherapy age-10.7 , 3.1 wt-not stated	multi-dose p.o	CL, $t_{1/2}$, Vd
Botha et al 1995	Indian/Blacks (South Africa) -epileptics	52	mixed therapy age-7.6 , 4.2 wt - 24.2 , 12.5	multiple dose po	CL, Vd, Ka

Although clearances of polytherapy patients of both population showed no distinct differences, clearances(95% confidence interval) of Japanese(0.010-0.015L/kg.hr) and coloured South African(0.018-0.024L/kg.hr) monotherapy patients proved otherwise. Nevertheless, this difference was not visible between studies of Chiba et al (1985) and Cloyd et al (1993).

4.4 Phenobarbitone

This antiepileptic drug is the oldest drug used in generalised and partial seizures. It is currently more widely used in neonatal seizures and the prevention of neonatal hyperbilirubinaemia. For this reason pharmacokinetic data concerning neonates and younger children were more available than older children or adults.

Phenobarbitone has not been reported to show ethnic differences in metabolism. However, Vessel and Page (1969) reported genetic control of

phenobarbital-induced shortening of plasma antipyrine half-lifes in man. Their studies involved examining the effect of phenobarbitone on single dose of antipyrine in four sets each of identical and fraternal twins. Their results showed that after a 2 week therapy of phenobarbitone(2 mg/kg) the half-lifes of antipyrine was reduced from 12.7 hours to 8.0 hours. Further results revealed greater similarity of inductive responses for identical than fraternal twins which then implicates a genetic factor in the regulation of the hypothesized inductive process. Their findings reported the contribution of hereditary to trait to be 99% which could probably explain interindividual differences in the activities of hepatic microsomal enzymes among individuals.

Table 4-9: Studies that examined the characteristic of neonatal phenobarbitone pharmacokinetics.

Reference	Neonates age group -no of patients	Dose (mg/kg) route	t_{max}(h) Mean,sd	C_{max}(g/L) Mean,sd	$t_{1/2}$ Mean,sd
Jalling et al 1974	Term - 10	5-13(IM)	6.4,3.6	12.9,4.0	108,48
	Term - 8	6-21(PO)	8.6,4.2	14.0,5.3	96,32
Royer et al 1976	Premature-12	10(IM)	40.1,17.	21.2,2.6	120,32
	Dysmature Prem - 3	10(IM)	0	18.3,2.4	74,40
	Dysmarure Term - 10	10(IM)	7.0,5.7	17.8,2.6	130,75
	Term - 5	10(IM)	31.2,14.8	16.0,3.3	93,31
			26.6,7.7		
Heiman & Gladtke 1977	Term - 14	5-10(IV)	-	-	119,16
	2-3 months-16	10(IV)			83,5
	4-12months - 14	10(IV)			63,4
	1-5 year - 7	10(IV)			69,3
Pitlick et al 1978	Term/Premature-32	20(IV)	-	-	67-115
Fischer et al 1981	Term (unasphyxiated) -5	14-21(IM)	-	-	110,63
	Term (asphyxiated) -10	13-20(IM)			100,44
Gillman et al 1983	Term/Premature -16	7-15(IV)	-	-	244,181
Kossman et al 1985	Term hyperb unasphyxiated-5	10(IM)	0.5,0.3	12.4,1.3	-
	Premature hyperb unasphyxiated -15		1.1,0.5	10.9,1.0	

The pharmacokinetics of phenobarbitone in neonates and young children has been reviewed by Battino et al (1995) and some of the findings are tabulated in table 4-9. Their findings found that the elimination of phenobarbitone is delayed in neonates and can be explained by the slow metabolism, limited renal excretion and the more acidic urine. These conclusion are based on results obtained by Kossman et al (1985), Heiman and Gladtke (1977), and Pitlick et al (1978) where the terminal half-lifes of term and premature neonates range from 67 to 409 hours respectively. These

values are higher than that are observed for older children and adults of 37 to 73 and 75 to 126 hours respectively [Levy et al 1991].

Factors that affect phenobarbitone elimination could also include duration of postnatal drug exposure, gestational age and neonatal asphyxia. However, the latter two factors were still inconclusive as results of other studies were found to be contradictory (table 4-9).

The effect of postnatal drug exposure is clearly demonstrated by Kossman et al (1985) where they observed the terminal half-lifes in premature infants during the first 4 to 5 days of life was low, but with an abrupt acceleration during the second week. Heiman and Galdtke (1977) observed the terminal half-lifes decreases from 119 ± 16 hours in term babies to 69 ± 3 hours in babies between the age of 1 to 5 years.

The differences in results on the effect of gestational age on the elimination of phenobarbitone can be highlighted by comparing the studies of Kossman et al (1985) and Nahata et al (1988) to that of Royer et al (1976), Jalling (1974), Gillman et al (1983), and Pitlick et al (1978). The former two studies found the trend in elimination decreases with age while the latter four studies found the differences were insignificant ($p > 0.05$).

The influence of neonatal asphyxia is also indecisive when the results by Gal et al 1984 showed the clearances of asphyxiated and non asphyxiated neonates(0.0041± 0.001 versus 0.0087 ± 0.0039 L/kg.hour) are significant while that Fischer et al (1981) found no such differences(0.0064 ± 0.0026 versus 0.0064 ± 0.0023 L/kg.hr)

Painter (1989) reviewed the elimination of phenobarbitone and noted that the rate was higher in children than in adults. This observation was however inconclusive since the studies used were mainly from young children age(n=2772) ranging from 0-18 years while that of adults(n=72) [Svensmark & Buchthal 1964, Duran et al 1988, Suganuma et al 1981, Yukawa et al 1992, Rossi et al 1979, Suzuki et al 1980] in single dose trials. Nevertheless, the progressively increased concentration/dose(C/D) ratio with age in some of these studies showed the possibility of age-dependent elimination rate.

The above results hypothesise that maturation changes in absorption lead to increase in C/D ratio but Rust and Dodson (1989) showed this was not true. Their

results showed that children and adults had in fact a similar absorption rate. C/D ratio was however affected by concomitant AED with the exception of valproic acid [Suganuma et al 1981, Yukawa et al 1992].

Reports on the correlation of dose and serum concentrations were highly variable and at times indecisive. This is partly due to the fact that some studies report the relationship is linear but not mentioning any statistical proof of its relationship. Nevertheless, Brachet-Liermain et al (1975) published result of a single dose study on infants and found the relationship is poorly related. Studies by Jalling (1974), Painter and Pippenger (1978) and Jalling (1975) on infants and neonates on chronic therapy showed the relationship is linear. Results on children showed the correlation is poor [Davis et al 1981, Mucklow et al 1981, Yukawa et al 1992] or significant [Melchior 1965]. Findings in adult patients showed that the relationship is linear [Martin et al 1979, Svensmark and Buchthal 1963]. Studies in mixed age population presented that the relationship is non-linear [Eadie et al 1977, Driessen and Hoppener 1977].

Table 4-10: Studies on the effect of age on serum concentration/Dose ratio in children

Reference	Age group(years) - sample size	Child:adult	Concurrent therapy	Dose(mg/kg)	Final conclusion
Svensmark & Buchthal 1964	1-14(49)	49:0	mixed	2-3	significant
Suzuki et al 1980	<1-38	848:24	mono/poly	1-10	significant between monotherapy and polytherapy
Suganuma et al 1981	<3 - 16	98:0	mono/poly	2-3	monotherapy, significant polytherapy-variable
Rossi et al 1979	0 - 7	92:0	mono	-	significant
Duran et al 1988	<3 - 19	474:62	mono	12	significant from <3-6, not significant from 7-19
Yukawa et al 1992	<2 -15	1226:0	mono/poly	2-6	Not significant with age

Abbreviation: Mono, monotherapy, Poly, polytherapy

Although the above studies reported either linear or non-linear relationship between serum concentration and dose, Eadie et al (1977) also reported the possibility of a curvilinear relationship from a mixed age population group. They further stated

that serum level fell progressively with increased age. These results clearly indicates the presence of intraindividual and interindividual variations among population.

4.4.1 Ethnic-based studies

Although phenobarbitone pharmacokinetics have been well established, ethnic-based studies were rare. To date, only five population studies reported the ethnicity of patients used [Peiris et al 1988, Duran et al 1988, Botha et al 1995a, Yukawa et al 1992a, Yukawa et al 1992b]. Two of these were Japanese-based while the other three were from Sri Lanka, Spain and South Africa. A summary of these studies were presented in table 4-11.

Table 4-11:Phenobarbitone population pharmacokinetics of various ethnic groups

Reference	Ethnic(Country)/ Patient-type	Sample size	Age(years) range or mean,se	Dose/ Route of administration	Pharmacokinetic parameters/Methods of analysis
Duran et al 1988	Spain (Caucasian) -epileptics	536	1-73	Multiple dose -retrospective	level/dose ratio
Peiris et al 1988	Holland (Caucasian) vs Sri Lankan	47:47	10-72	multiple dose -prospective	serum concentration, relative serum concentration(RSC)
Yukawa et al 1992a	Japan (Japanese) -epileptics	539	paediatric + adult	Multiple dose -retrospective	level/dose ratio
Yukawa 1992b	Japanese (Japan) -epileptics	539	paediatric + adult	Multi-dose -retrospective	-Css, CL and serum level and dose relationship -Non-linear mixed effect model(NONMEM)
Botha et al 1995	Blacks/Indian (South Africa) -epileptics	32	5.5 , 3.2	Multi-dose -retrospective	-Css, CL and serum level and dose relationship -Non-linear mixed effect model(NONMEM)

Reports on level/dose ratios among the paediatric patients varied between population. Sagunuma et al (1981) and Yukawa E (1992a) observed significant level/dose ratios with age in the Japanese population in the age range of 0 to more than 15 years. Duran et al (1988) however found the same degree of significance only for the patients age less than 3 to 6 years old. The latter results were however similar to that reported by Rossi et al (1979), Guelen et al (1975), Benedetti et al (1979),

Minagawa et al (1981) and Davis et al (1981) which studied patients with undisclosed ethnic background between the age-group of 2 months to 6.5 years. These findings could possibly be due to interethnic differences in metabolism between paediatric Caucasian, Japanese and coloured South African children.

Peiris et al (1988) however noted although the coefficient of variation for relative serum clearance for the Sri Lankan patients was higher than their Dutch counterparts, these differences did not reach any statistical significance.

The studies by Yukawa (1992b) and Botha et al (1995a) estimate population clearance from routine data using the NONMEM method developed by Sheiner et al (1977) and Beal and Sheiner (1980). Both Botha et al (1995a) and Yukawa et al (1992b) also showed that clearance was significantly affected by weight. The latter further concluded that this relationship was most affected by patients on polytherapy with other AED's than those combined with sodium valproate.

Although the population ages of Yukawa (1992b) and Botha et al (1995a) were distinctly different, their clearances(95% confidence interval) were similar; monotherapy(5.5-7.6L/kg.hr and 6.2-9.0L/kg.hr), polytherapy with valproate(3.7-4.7L/kg.hr and 4.0-6.0L/kg.hr) and polytherapy with other AED's(5.0-6.0L/kg.hr and 5.6-8.0L/kg.hr) patients group(table 3.12). Their mean intraindividual(18.65%,18.1%) and interindividual(20.07%,18.0%) variation were almost identical. These findings must still be examined further because of the different population age-groups studied. Botha et al (1995a) also noted the possibility of no interethnic differences of paediatric Indians and Black patients in South Africa as their mean clearances were significantly similar..

Significant clearance and age relationship had actually been observed earlier by Grasela and Donn (1985) on neonatal patients between the ages of 24 to 42 weeks. Their results showed that mean clearances values of neonatal patients were more than 10 times lower(0.0047L/kg.hr) than those in the paediatric or adults age.

4.6 Review limitations

This review suffers from the lack of published ethnic-based studies. This problem mainly arise from researchers who find that describing the racial or ethnic composition of patients being assumed as clinically unimportant or possibly

discriminatory. Reports from the developing or third world countries are also too few for a full assessment of clinical applicability and relevance in all ethnic groups.

Although evidence about interethnic differences of certain drugs such as phenytoin had already been mentioned and reviewed, for drugs such as carbamazepine, valproic acid and phenobarbitone, where this relationship has yet to be determined, the problems of establishing evidence is almost impossible. Hence to substantiate or dissaprove the influence of ethnicity on the pharmacokinetic of these three establish antiepileptic drugs are made difficult by the scarcity of published data.

The limited number of studies cited were again restricted due to problems of inter-study variations. Inter-study variations can be broadly categorised into three main types. First was the age group; paediatric(0-18 years), adult(>18 years) and mixed(all ages), second the type of pharmacokinetic model employed and finally the type of computer software(OPT and NONMEM) used to estimate the population pharmacokinetics. These inter-study variations reduced the number of studies for comparison which makes any conclusion indecisive.

4.7 Conclusion

Establishing ethnic-based population pharmacokinetics for established antiepileptic drugs may prove important in optimizing drug therapy. Inter-ethnic differences are evident in drugs such as phenytoin and carbamazepine but these differences are less clear cut for valproic acid and phenobarbitone.

Pharmacokinetic parameters which are associated with phenytoin inter-ethnic differences are its metabolic rate constant(*Km*), maximum metabolic rate(*Vmax*), half-lifes($t_{1/2}$) and clearance(CL). Half-lifes and clearance were the two main pharmacokinetics parameters linked to inter-ethnic differences in carbamazepine, valproic acid and phenobarbitone.

Reliability of conclusions made in this review was however restricted by the limited numbers of published ethnic-based literatures and inter-study variations. These findings otherwise showed inter-ethnic differences could be present for established antiepileptic drugs and more research is needed to verify their importance.

Part 2

Data extraction

Chapter 5

Methods and material

5.1 Background information

Data on epileptic patients were gathered retrospectively from government hospitals in Malaysia. The population data consisted of both paediatric and adult patients. The patients were mainly of Malay, Chinese and Indian origins which form the three most dominant ethnic groups in Malaysia.

Malays are called as the 'bumiputera's or original native of the land and this include other indigenous groups(such as Iban, Melanau) from Sabah and Sarawak(East Malaysia) from the island of Borneo. The Chinese population which came to Malaysia during the British colonisation period are mostly from the southern part of mainland China. Similarly, Indians are from the highly populated and heterogenous continent of India. A brief outline of the composition of the Malaysian multi-ethnic population and health system is described below.

5.1.1 Malaysia the country

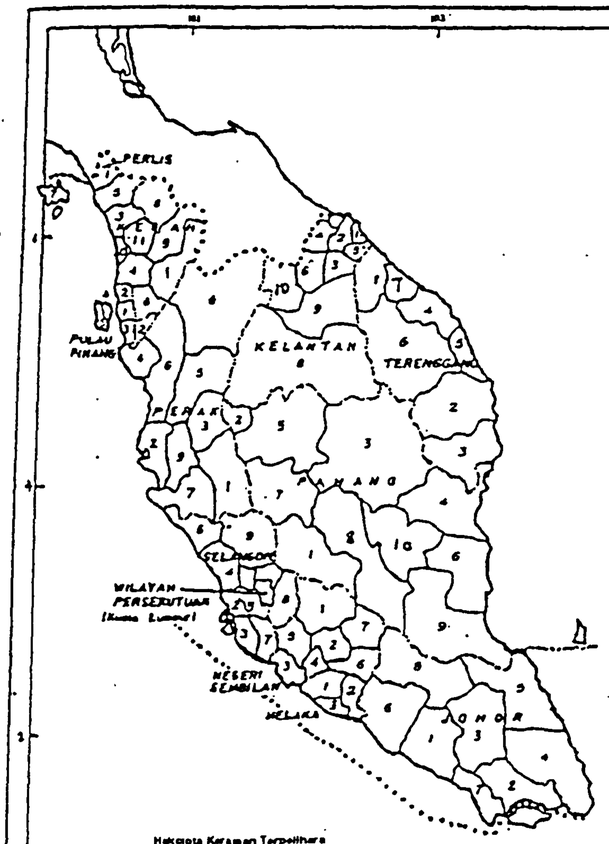
Malaysia is a tropical country situated 7 degrees north of the equator in the heart of South-east Asia. It comprises Peninsula Malaysia and the two states of Sabah and Sarawak on the island of Borneo. Together it covers a total area of 329,758 square kilometers(figure 5-1).

Malaysia comprises of thirteen states and two federal territories. The two federal territories are namely the capital city of Kuala Lumpur and the Island of Labuan. Nine of the states have a hereditary ruler respectively from which the Supreme head of State, the Yang Di-Pertuan Agong(King) is elected every five years [Malaysian Tourism Agency 1996].

Malaysia is a multiracial country with a population of approximately 19.9 million. This consists of the main ethnic groups of Malays(61.28%), Chinese(27.56%) and Indians(7.83%) and a very diverse group of indigenous people in Sabah and Sarawak. Table 5.1 and table 5.2 presented the population distribution by race and sex as in the year of 1994.

M A L A Y S I A

PETA MENUNJUKKAN NEGERI DAN DAERAH PENTADBIRAN
MAP SHOWING STATES AND ADMININSTRATIVE DISTRICTS



JOHOR
1. Batu Pahat
2. Johor Bahru
3. Kluang
4. Kota Tinggi
5. Mersing
6. Muar
7. Pontian
8. Segamat

KEDAH
1. Baling
2. Bandar Baharu
3. Kota Setar
4. Kuala Muda
5. Kubang Pasu
6. Kulim
7. Langkawi
8. Padang Terap
9. Sik
10. Yan
11. Pendang

KELANTAN
1. Bachok
2. Kota Bharu
3. Machang
4. Pasir Mas
5. Pasir Putih
6. Tanah Merah
7. Tumpat
8. Gua Musang (Ulu Kelantan)
9. Kuala Krai
10. Jeli

MELAKA
1. Utara (Alor Gajah)
2. Selatan (Jasin)
3. Tengah

NEGERI SEMBILAN
1. Jelebu
2. Kuala Pilah
3. Port Dickson
4. Rembau
5. Seremban
6. Tampin
7. Jempol

PAHANG
1. Bentong
2. Cameron Highlands
3. Jerantut
4. Kuantan
5. Lipis
6. Pekan
7. Raub
8. Temerloh
9. Rompin
10. Maran

PULAU PINANG
1. Tengah (Bukit Mertajam)
2. Utara (Butterworth)
3. Selatan (Nibong Tebal)
4. Timur Laut
5. Barat Daya

PERAK
1. Batang Padang
2. Manjung (Dinding)
3. Kinta
4. Kerian
5. Kuala Kangsar
6. Larut dan Matang
7. Hilir Perak
8. Ulu Perak
9. Perak Tengah

PERLIS
Pekan

SELANGOR
1. Gombak
2. Klang
3. Kuala Langat
4. Kuala Selangor
5. Sepang
6. Sabak Bernam
7. Selayang
8. Ulu Langat
9. Ulu Selangor

TERENGGANU
1. Besut
2. Dungun
3. Kemaman
4. Kuala Terengganu
5. Marang
6. Ulu Terengganu
7. Setiu

SABAH
1. Tawau
2. Lahad Datu
3. Semporna
4. Sandakan
5. Kinabatangan
6. Beluran (Labuk & Sugut)
7. Kota Kinabalu
8. Ranau
9. Kota Belud
10. Tuaran
11. Penampang
12. Papar
13. Kudat
14. Kota Marudu
15. Pitas
16. Beaufort
17. Kuala Penyu
18. Sipitang
19. Tenom
20. Nabawan (Pensangan)
21. Keningau
22. Tambunan
23. Labuan
24. Kunak

WILAYAH PERSEKUTUAN

SARAWAK
1. Kuching
2. Bau
3. Serian (Ulu Sadong)
4. Simunjan (Hilir Sadong)
5. Lundu
6. Sri Aman (Simanggang)
7. Lubok Antu
8. Betong (Saribas)
9. Saratok (Kalaka)
10. Sibu
11. Mukah
12. Kanowit
13. Daiet
14. Miri
15. Bintulu
16. Marudi
17. Limbang
18. Lawas
19. Sarikei
20. Maradong
21. Daro
22. Julau
23. Kapit
24. Song
25. Belaga
26. Samarahan
27. Tatau

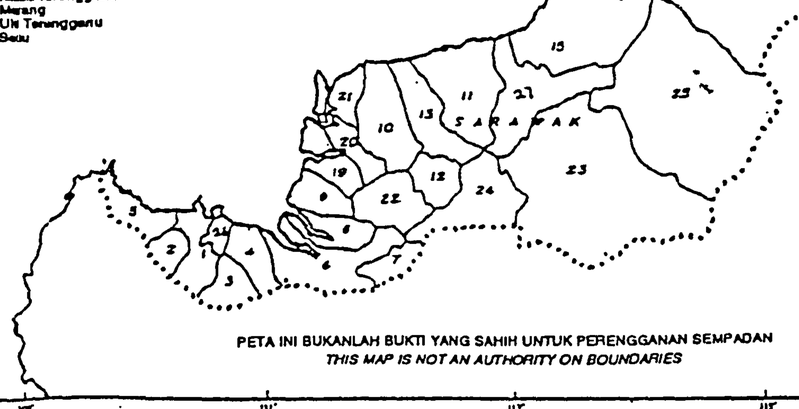
PETUNJUK
REFERENCE

Sempadan Antarabangsa
International Boundary

Sempadan Negeri
State Boundary

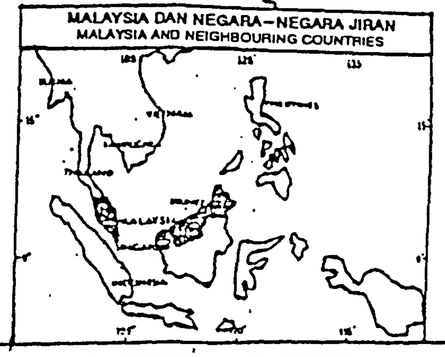
Sempadan Daerah
District Boundary

0 100 200 300
KILOMETER (KILOMETRES)



PETA INI BUKANLAH BUKTI YANG SAHIH UNTUK PERENGGANAN SEMPADAN
THIS MAP IS NOT AN AUTHORITY ON BOUNDARIES

MALAYSIA DAN NEGARA–NEGARA JIRAN
MALAYSIA AND NEIGHBOURING COUNTRIES



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Prepared and published by the Department of Statistics, Malaysia

Diterbitkan dengan kebenaran Pengarah Pemetaan Negara, Malaysia.
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(DNMM 24. 04. 1/Jld. 23/75)

Dicetak oleh Jabatan Perangkaan, Malaysia
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Table 5-1: Mid year population estimates by ethnic groups and state, Malaysia, 1994

State	Total(,000)	Malayan citizen							Non-citizen
		Total Malaysian citizen	Total	Bumiputera	Other Bumiputera	Chinese	Indian	Others	
Johor	2310.6	2250.3	1152.3	1141.3	11.0	801.2	148.9	148.9	60.4
Kedah	1453.3	1446.4	1080.9	1079.3	1.5	235.5	105.8	105.8	6.9
Kelantan	1313.2	1287.4	1214.6	1205.6	9.0	56.6	6.0	10.2	25.8
Melaka	562.7	555.5	324.2	321.4	2.8	184.0	39.1	8.2	7.2
N.Sembilan	764.0	753.4	399.4	391.5	7.9	220.0	123.2	10.8	10.6
Pahang	1154.7	1121.1	828.9	789.0	39.9	208.8	67.4	15.9	33.7
Perak	2081.5	2067.8	1003.7	962.2	41.4	732.5	281.7	50.0	13.7
Perlis	201.4	199.4	166.2	165.6	0.6	24.0	3.7	5.4	2.0
P.Pinang	1171.2	1153.4	448.0	446.7	1.3	572.2	127.0	6.2	17.8
Selangor	2591.4	2511.2	1173.8	1157.4	16.4	800.0	407.9	129.5	80.2
Terengganu	874.9	854.2	817.4	815.9	1.6	30.1	2.8	4.0	20.7
K.Lumpur	1304.5	1245.8	501.0	496.6	4.4	582.0	145.4	17.5	58.6
Sabah	1977.0	1483.1	1074.2	782.9	291.3	221.1	9.3	178.5	493.9
Sarawak	1837.8	1818.8	1298.2	1186.4	111.8	504.2	5.0	11.4	19.0
Labuan	59.6	48.8	35.4	27.2	8.2	9.0	0.9	3.5	10.8
Total	19657.9	18,796.5	11,518.2 (61.3%)	9501.2	2017.0	5181.3 (27.6%)	1471.1 (7.8%)	622.9 (3.3%)	861.4

States in bold are states where the hospitals are situated

Table 5-3 : Distribution of hospitals and special medical institutions by state and by bed strength, Malaysia 1994

State	Regional Hospitals	District hospitals	Overall beds	Special Med. Inst.	Institution beds
Perlis	1	-	404	-	-
Kedah	1	7	1917	-	-
Pulau Pinang	1	4	2179	-	-
Perak	1	12	3794	1(Mental)	3000
Selangor	1	5	1538	1(Leprosy)	836
Fed.Territories	1	1	2599	1(Tubercolosis)	116
Negeri Sembilan	1	4	1336	-	-
Melaka	1	1	910	-	-
Johor	1	8	2553	1(Mental)	2080
Pahang	1	7	1536	-	-
Terengganu	1	3	1093	-	-
Kelantan	1	7	1344	-	-
Sabah	3	12	2505	1(Mental)	302
Sarawak	1	17	2826	1(Mental, Leprosy)	378
Malaysia	16	89	26538	7	6712

Table 5-2: Mid-year population estimates by sex and state,1994[a]

State	Total(,000)	Sex	
		Male(,000)	Female(,000)
Malaysia	19,657	9,975.90	9,682.00
Johor	**2,310.6**	**1,181.0**	**1,129.6**
Kedah	1,453.3	719.0	733.6
Kelantan	1,313.2	648.8	664.4
Melaka	562.7	277.2	285.5
Negeri Sembilan	764.0	385.5	378.5
Pahang	1,154.7	598.3	556.4
Perak	**2,018.6**	**1,033.7**	**1,047.9**
Perlis	201.4	101.0	100.4
Pulau Pinang	**1,171.2**	**582.3**	**588.9**
Sabah	1,977.0	1,040.6	936.4
Sarawak	1,837.8	944.2	893.6
Selangor	**2,591.4**	**1,317.4**	**1,274.0**
Terengganu	874.9	445.3	429.6
Federal Territory (Kuala Lumpur)	1,304.5	669.0	635.5
Federal Territory (Labuan)	59.6	31.9	27.7

a As at 30th June, 1994. The figures are estimated using the 1991 census data as the base and adding the annual natural increase, that is, the excess of births over deaths

States in bold signify where the hospitals are situated and used in this study

5.1.2 The Health System

Each of the thirteen states and two federal territories provide both inpatient and outpatient care programs which form a vital part of the medical care program of the Malaysian Ministry of Health. This program is provided through a network of sixteen general hospitals, eighty-nine district hospitals and four mental institutions throughout the country [Annual Report, Ministry of Health, Malaysia 1991]. Table 5-3 showed the distribution of hospitals and special institutions by state and bed strength.

5.2 Research protocol

The protocol involved visits to hospitals with a special emphasis on hospitals which provide therapeutic drug monitoring services on the four antiepileptic drugs. Approval from the Director General of Health, Ministry of Health, Malaysia, was initiated. Upon approval, patients profiles were obtained from case-notes dated to the time when drug monitoring was first initiated. Only complete case-notes were taken and lost notes were omitted. Summary of the proposed research protocol in figure 5-2.

Figure 5-2: Research protocol for the multi-centre retrospective study.

1. Hospitals :

(a) General Hospitals

(b) Psychiatric Hospitals

⇓

2. Patient selection

⇓

3. Data collection:

(a) Pharmacy department

(b) Biochemistry Department

(c)Outpatient's clinics

(d) Records Department

⇓

4. Organisation of Data

⇓

5. Data analysis

5.3 Data retrieval

The period allocated for data collection was approximately three months. The dates for hospital visits were from the 30 September to the 20 December 1997. Detailed outline of dates for each hospital visit is in appendix 5-1. Since patients' files were kept in the record office, the relevant clearance from the proper department was obtained. The departments involved were the pharmacy department, biochemistry department, record office, and the neurology, medical or psychiatric units. All collected data were transferred to the standardised worksheet (appendix 5-2) and were kept confidential and no name of patients were recorded.

Data from the years of 1993 - 1996 were retrieved from five government hospitals which offer therapeutic drug monitoring services for these drugs. The hospitals selected are those where monitoring of the drugs is done routinely by the pharmacy department where control, recording, accessibility and retrieval of patient's data are better organised. The protocol for therapeutic blood monitoring(appendix 5-3a,5-3b) is similar to the guidelines outline in the Ministry of Health Handbook 1996.

5.4 The Hospitals

The five selected hospitals selected were Hospital Sultanah Aminah Johor Bahru, Hospital Ampuan Rahimah Kelang, Hospital Ipoh and Hospital Pulau Pinang which are general hospitals, and Hospital Permai Johor Bahru which is a mental institution dealing mainly with psychiatric cases. These hospitals spread out all over the peninsula of Malaya and were specifically identified because of several reasons. The first is that these hospitals are situated in four of the most populated states(bold letters) of Malaysia and the demographic statistic of the four states is in table 5-1. The four states concern were Johor, Selangor, Perak and Penang. Together these states constitute approximately 41.5% of the total population. However the inter-ethnic distribution were slightly different. Malays were more prominent in Johor, Chinese in Pulau Pinang, while Indians was highest in Selangor. Population estimates by sex is in table 5-2 [State Data Bank, Department of Statistic 1994, Social Statistic Bulletin, Department of Statistic 1974]. Overall, the ratio per 100 of male to female of the four selected states was almost equivalent, i.e., 50.3:49.7. This is similar to the national ratio of 50.7:49.3.

Secondly the combined bed capacity of the five hospitals of about 6260 would provide enough patient data during the screening procedure. Types of services offered were similar for all hospitals with the exception of Hospital Permai which is an institution specialised in mental health. A summary of the services that are available in each of the five hospitals is in Table 5-4 [Annual report Hospital Sultanah Aminah, JB, Hospital Permai, Hospital Klang, Hospital Ipoh, Hospital Penang]

Thirdly, the hospitals selected are all classified as either regional or specialised hospitals. Regional Hospitals are hospitals where patients come for treatment and review from several catchment areas. Specialised hospitals are institutions where treatment are specific towards certain disease condition such as epilepsy. Regional Hospitals are referral hospitals with medical consultants of various specialities providing services for patients coming from smaller district hospitals. District hospitals are hospitals with only a few or no medical consultants managing the hospitals. These hospitals usually have a bed capacity ranging from 100 to 400 beds. Each regional hospital services at least five district hospitals. An example of a regional hospital is Hospital Sultanah Aminah Johor Bahru which services six district hospitals in the state of Johor. Other than Hospital Sultanah Aminah, therapeutic drug monitoring is practiced quite extensively in Hospital Tengku Ampuan Rahimah, Selangor, Hospital Ipoh, Perak, Hospital Alor Star, Kedah, Hospital

Kota Bahru, Kelantan, Hospital Besar Kuala Terengganu, Terengganu, Hospital Kuantan, Pahang, Hospital Queen Elizabeth, Sabah and Hospital Kuching Sarawak.

There are currently four specialised hospitals in Malaysia. All four hospitals are hospitals which handles mainly mental health care. Two of the hospitals are based in West Malaysia and one each are situated in Sabah and Sarawak in East Malaysia. Therapeutic drug monitoring is routinely practiced in two of the hospitals, Hospital Permai and Bahagia in West Malaysia.

These hospitals were also selected due to all patients were treated and reviewed by qualified physician. These hospitals follow stringent guidelines(based on Ministry of Health instruction) for blood sampling with experienced paramedical staffs managing the department.

Finally, the period and cost of study had limited the number of hospitals to be visited. It should be mentioned that the period of study is limited to three months only and these hospitals are situated from the southernmost state(Johor) of the Malay peninsula to the northern state of Penang. The distance between these two states is already about 1120 kilometers and if hospitals from the States of Sabah or Sarawak(Borneo Island) were to be included(more than 1600 kilometers from Johor), the time is surely inadequate and substantial extra cost would have been incurred which could not be met from within the study budget.

5.5 Selection of patients

Patients were initially screened from data recorded at the Therapeutic Drug Monitoring Unit of the Pharmacy Department. This was then cross-checked at the department concerned, i.e. medical, neurology etc. The final list of patients recruited was selected on the basis that there were complete written records and they were taking the drugs orally for at least one month. Complete written record were notes made by the physician concerning patient history, biodata, examination, observation, and conclusion. Patients with missed but retrievable records were included. The criteria for selection of population sample were as follows:

a. Inclusion criteria:

i. Patients who must be diagnosed and confirmed to be epileptics.

ii. Patients receiving at least one of the study drugs.

iii. All laboratory results for serum levels must use a known appropriate analytical methodology

iv. Patients must be on the studied drugs for at least a month before any monitoring is done

b. Exclusion criteria:

i. Patients which were non-compliant

ii. Patients with incomplete records

iii. Etiology of seizure is neoplastic, progressive degenerative, metabolic, demyelinating, or active infection. (Patients with a previously resected tumor that is not recurrent are accepted)

iv. Seizures due to acute medical disorders such as hypoglycemia, uremia etc are not considered

v. Seizures due to alcohol abuse or alcohol withdrawal

vi. Seizures due to neurologic disorder or any kind of serious medical disorder or active infection.

Due to the distance between hospitals and the short study period only five hospitals were visited although seven hospitals were initially targeted. Retrieval of data was often tedious and time consuming since none of the hospitals were computerised and data had to be searched by hand. Details of data collected were summarised by the format in appendix 5-4.

Patients were selected only if they were on antiepileptic drug therapy for at least a month and were confirmed epileptics with no known metabolic or neurological disorder. These were checked for non-compliance based on the written notes or comments made in the therapeutic request forms. However, no formal interview with patients were made to confirm the findings.

No age limit was set and patients were grouped in their respective ethnic background. Patients were categorised as adults if their age is 18 years or more and those below this age limit are considered in the children group.

The number of patients screened and selected were 696, 134, 397, 180 and 461 for hospital Johor Bahru, Hospital Permai, Hospital Kelang, Hospital Ipoh, and Hospital Penang respectively. However, the number of suitable data were 1,215 or 65.04 percent of the total number shortlisted. The number of adult to children was 874 and 341 with the most number of patients(394) selected coming from Hospital Johor Bahru. Reasons for exclusion for about 34.96% of the shortlisted patients were inadequate patient's data, non-compliance and lost files. Inadequate patient's data means that patients notes were incomplete for a full compilation as that is listed in appendix 5-4.

Table 5-4 and 5-8 highlighted the demographic and clinical data of children and adult epileptic patients of each individual hospital visited. Patients were categorised into epilepsy types namely generalised(code 1), partial(code 2) or others(code 3). Epilepsy categorised as others include specific epileptic syndromes and unclassified epilepsies. Examples of specific epileptic syndromes are febrile convulsions, reflex epilepsies and seizure induced seizures while unclassified epilepsies are neonatal seizures and nocturnal tonic-clonic seizures.

Table 5-4 : Patients' characteristic of Hospital Sultanah Aminah, Johor

Variables	Malay	Chinese	Indian
Sample size	194	117	83
Sex(M:F)	80:114	70:47	37:46
Age group (Children:Adult)	73:121	63:54	51:20
Mean Age(years),sd	24.52,14.59	21.42,13.49	26.08,12.48
Range	2-67	2-53	2-55
Mean Weight(kg),sd	48.27,19.23	45.87,19.22	13.14,17.82
Range	7.90-84	9.60-81.75	9.50-79
Length of disease(years),sd	9.88,9.15	9.61,8.35	9.66,8.12
Range	0.25-33	0.50-39	0.75-40
Epilepsy types(1:2:3)	120:26:48	71:15:31	53:15:15
Therapy -mono:poly	126:68	70:47	57:26

Abbreviation: mono=monopoly, poly=polytherapy

Seizure index was the indicator and the common metric used for outcome measurement of seizure control. Seizure index was calculated by dividing the number of seizures per day. The record of seizures were obtained from the written notes on the day of hospital visit. For example, if a patient has a history of 5 seizure per three months;

$$\text{seizure index} = \frac{\text{number of seizures}}{\text{period of observations}} = 5/90 = 0.0555(\text{seizures/day})$$

Patients were then grouped into controlled or uncontrolled groups.

Table 5-5 : Patients' characteristic of Hospital Permai, Johor

Variables	Malay	Chinese	Indian
Sample size	51	59	29
Sex(M:F)	30:21	27:32	23:6
Age group (Children:Adult)	51:0	59:0	29:0
Mean Age(years),sd	41.00,12.43	39.85,12.05	42.99,15.61
Range	21-71	17-69	19-81
Mean Weight(kg),sd	60.92,9.10	59.06,9.14	59.01,8.35
Range	46-75	41-75	50-72
Length of disease(years),sd	10.26,8.29	9.97,8.09	11.64,10.96
Range	1-34	0.50-42	0.25-42
Epilepsy types(1:2:3)	47:4:0	55:4:0	26:3:0
Therapy -mono:poly	12:39	20:39	9:20

Abbreviation: mono=monopoly, poly=polytherapy

Table 5-6 : Patients' characteristic of Hospital Klang, Selangor

Variables	Malay	Chinese	Indian
Sample size	86	48	115
Sex(M:F)	44:42	19:29	48:67
Age group (Children:Adult)	38:46	7:37	43:72
Mean Age(years),sd	26.52,18.11	33.09,14.93	25.37,12.91
Range	1-73	2.60-62	2.75-50
Mean Weight(kg),sd	44.26,18.39	52.15,15.73	48.13,15.72
Range	5.10-84	11-87	13-89
Length of disease(years),sd	8.60,8.94	16.52,11.80	10.96,9.45
Range	1-36	2-50	1.50-33
Epilepsy types(1:2:3)	61:22:3	40:8:0	102:12:1
Therapy -mono:poly	52:34	10:38	67:48

Abbreviation: mono=monopoly, poly=polytherapy

Table 5-7 : Patients' characteristic of Hospital Ipoh, Perak

Variables	Malay	Chinese	Indian
Sample size	57	26	51
Sex(M:F)	28:29	16:10	21:29
Age group (Children:Adult)	25:32	6:20	15:36
Mean Age(years),sd	21.59,15.39	28.71,13.17	24.43,13.02
Range	3-61	4-55	3-58
Mean Weight(kg),sd	44.88,20.56	53.65,14.72	47.98,15.77
Range	11-80	16-80	13-82
Length of disease(years),sd	10.61,6.86	13.24,8.52	12.67,8.94
Range	2.50-30	3-32	1.50-40
Epilepsy types(1:2:3)	53:4:0	23:3:0	44:7:0
Therapy -mono:poly	28:29	10:16	38:13

Abbreviation: mono=monopoly, poly=polytherapy

Table 5-8 : Patients' characteristic of Hospital Penang, Penang

Variables	Malay	Chinese	Indian
Sample size	82	173	44
Sex(M:F)	40:42	91:82	26:18
Age group (Children:Adult)	15:57	28:145	30:6
Mean Age(years),sd	29.33,12.95	34.06,15.83	33.82,13.24
Range	2-55	6-94	9-55
Mean Weight(kg),sd	53.57,16.92	55.97,12.40	54.41,16.18
Range	12-90	16-80	23-82
Length of disease(years),sd	15.11,11.86	13.65,11.83	18.05,10.83
Range	1.75-46	1-61	2-39
Epilepsy types(1:2:3)	53:24:5	125:43:5	29:14:1
Therapy -mono:poly	34:48	60:113	18:26

Abbreviation: mono=monopoly, poly=polytherapy

5.6 Therapeutic Drug Monitoring

Therapeutic Drug monitoring is one of the activities of the Clinical Pharmacokinetics Services [Pharmaceutical Services Division, Ministry of Health Malaysia, 1996]. It was first started in the mid-eighties by Hospital Pulau Pinang and upto 1995, more than forty hospitals had started the service. The range of therapeutic blood monitoring depends largely on the hospital needs and financial capabilities. This service is of clinical importance as demonstrated by the many departments utilising its services.(table 5- 9)

i. Guidelines for Therapeutic Drug Monitoring.

A standard guideline is used for all routine blood sampling [Pharmaceutical Services Division, Ministry of Health 1996]. Requests for therapeutic blood monitoring are restricted to patients reviewed by either consultants or senior medical officers. These guidelines stress the importance that hospitals should follow stringent rules for blood sampling with experienced paramedical staff managing the department.

The guideline outlines that patients must be informed of the specified date upon which blood sample will be taken. This is usually a week before the next review date. This would ensure that the result(s) can be made available on the schedule date of review.

Table 5-9 : Summary statistic of Therapeutic Drug Monitoring services in five main hospitals in Malaysia (1995)

	Hospital				
Facility	**J.Bahru**	**Permai**	**Kelang**	**Ipoh**	**Penang**
Bed	989	2080	811	990	1390
TDM unit	y	n	y	y	y
Test/year	4898	700	1838	1221	5234
% test/year(AED	45	95	43	27	46
% test/year (antibiotics)	46	<1	32	46	43
Others	9	4	25	27	11
Departments					
-Surgery	y	n	y	y	y
-Medical	y	n	y	y	y
-O & G	y	n	y	y	y
-Paediatric	y	n	y	y	y
-Anaesthiology	y	n	y	y	y
-Opthalmology	y	n	y	y	y
-Psychiatric	y	y	y	y	y
-ICU	y	n	y	y	y
-CCU	y	n	y	y	y
-Outpatient	y	y	y	y	y
-A&E	y	n	y	y	y

Abbreviation:
y = yes, n = no, O & G = Obstetric & Gynaecology, ICU= Intensive Care unit, CCU = Coronary Care Unit, A&E= Accident & Emergency Department
Antibiotics, are the aminoglycosides i.e. gentamicin, amikacin, netilmicin, vancomycin
Others, include theophylline, digoxin, cyclosporin

Blood sample was taken between 10.00am and 12.00am where 3-5 milliliters of blood are extracted by venuspuncture and placed into a 10 ml test-tube. The test-tube was

then labeled and sent to the Pharmacy department with an accompanying request form about details of patient biodata and drug therapy(appendix 5-5). The samples were sent on the same afternoon to be analysed. Results of the assays were obtained either on the same or following day.

ii. Analytical procedures

The fluorescent polarization immunoassay(FPIA) is used to determine serum concentration. The coefficient of variation for the four analysed drugs is between 2 to 4%. FPIA is ranked between good and excellent for all drug tests and with a high work rate. However, its usefulness is limited to tests involving plasma or serum sample only. A summary of the relative advantages and disadvantages of FPIA over the other analytical methods are in appendix 5-6 to 5-9 [Winter & Tozer 1991, Levy et al 1991].

iii. Drugs Assay

The two most common groups of drugs monitored are the aminoglycoside antibiotics and antiepileptics such as phenytoin, carbamazepine, valproic acid and phenobarbitone [unpublished report from the Ministry of Health, Pharmacy Division, Malaysia 1995]. Table 5-9 summarised the range and extent of therapeutic drug monitoring activities of these four drugs in Hospital Sultanah Aminah, Johor Bahru, Hospital Pulau Pinang, Penang, Hospital Ampuan Rahimah, Kelang, Hospital Ipoh, Perak, and Hospital Permai, Johor for the year 1995. Therapeutic drug monitoring services is offered for both in and outpatient in all hospitals with the only exception of Hospital Ipoh where patients are hospitalised whenever therapeutic drug monitoring is initiated.

5.7 Pharmacokinetics Models and statistical analysis

5.7.1 Individual patient data

The three main variables investigated were dose, serum concentration and clearance. Dose can either be in milligrams per day or milligrams per kilogram body weight. Serum concentration are converted to milligram per litres.

Since many patients with one or more doses having several measured serum concentrations, the average of the measured levels and the subsequent treatment

response(seizure index, i.e, controlled or uncontrolled) for each dose were taken. Many patients were also on two or more different dosage regimens on all three antiepileptic drugs(table 5-10).

Table 5-10 : Summary of dose-serum level pairs of patients on phenytoin, carbamazepine, valproic acid and phenobarbitone

Dose-serum level pairs	Phenytoin(n) -child:adult	Carbamazepine(n) -child:adult	Valproic acid(n) -child:adult	Phenobarbitone(n) -child:adult
1	17:259	51:210	70:99	28:67
2	35:277	79:139	129:115	19:55
3	12:80	10:37	31:22	7:11
4	2:19	0:6	12:14	0:3
5	0:8	0:2	1:1	-
6	-	0:1	-	-

The efficacy of the therapeutic or target range of phenytoin, carbamazepine, valproic acid and phenobarbitone was determined by the used of odd-ratios between seizure controlled and uncontrolled patients. The method essentially utilised only one single dose and serum concentration data from each patient and the details will be described in chapter 6. Random selection of a single dose/serum concentration pair from patients with more than one dose and serum concentration pairs were computer generated(appendix 5-10). This method of systematic selection was mainly employed to avoid inter-patient bias.

The same set of data was used to establish relationship between dose with either serum concentration or clearance of carbamazepine, valproic acid and phenobarbitone. However, two dose and serum concentration pairs were randomly selected from each individual during the determination of the Michealis-Menten constants for phenytoin. The computer based selection is described and presented in appendix 5-11. The formula used in calculating clearance and the Michealis-Menten constant are describe in section 5.7.2.

Both linear and multiple regression technique was used for analysis. Linear regression was appropriate because of its characteristic of being able to recognise the relationship of an dependent and independent variable. This relationship was summarised by the regression equation consisting of the slope and the intercept. The method also enable the test of significance and confidence intervals of hypothesis and make assumptions on the underlying errors on the test of significance. Multiple regression

methodology is also used in looking for relationship between continuous variables, and allowing for a third variable. It is also important when adjustment may need to be made for differences in confounding factors between groups. Data was analysed using the software available in the Minitab Statistical package, version 10.2.

5.7.2 Pharmacokinetic variables

Carbamazepine, sodium valproate, and phenobarbitone clearance was calculated on the assumption that at steady state(at equilibrium) the ratio of change is proportionate to the ratio of change of serum concentrations. Clearance is usually measured in liters per day (L/day) or liters per kilogram per day. (L/kg.day) The formula and assumptions by Wagner (1965) and is described in chapter 1, section 1.5.5 was used in calculating the clearance;

$$Css = \frac{F.D}{V_d.Ke.\tau}$$

where *C*ss is the average asymptomatic blood concentration, *D* is the dose, τ is the length of the dosage interval, *Ke* is the first-order rate constant for overall loss of drug from the blood, *F* is the fraction of each dose which is absorbed(bioavailibility factor), and *Vd* is the apparent volume of distribution.

Since clearance (*CL, Liters/day*) is equal to *Vd multiplied by Ke* , the formula can be rewritten as;

$$Clearance(CL) = \frac{F.D}{C_{ss}.\tau}$$

τ can be in minutes or hours per day. For simplicity, F is taken as equal to 1.

or $$C_{ss} = \frac{F.D}{Cl.\tau}$$

The regression equation was obtained by plotting the relationship of steady-state serum concentration(*Css*) and dose(*D*). For simplicity, *F* is taken as equal to 1 and τ is

taken as per day. This assumption is also based on the reason that the drugs taken by all patients were the same since the drugs came from a single government body.

As for phenytoin, assuming steady-state condition, the following linearised Michealis-Menten relationship(as described in chapter 1, section 1.5.5) was used to obtain clearance, *Vmax* and *Km*.

$$(S.F.R)_{ex} = \frac{Vmax - Km.[S.F.R.]}{C_{ss}}$$

where $(S.F.R)_{ex}$ is the final expected rate of absorption, which is dependent on the salt factor(*S*) form and is equal to 1, *F* is the bioavailibility while *R* is the rate of administration(dose/day) [equal = 1]. For this study, bioavailibility was assumed to similar to all patients for all patients and this omitted. *Vmax* is defined as the maximum rate of metabolism (metabolic capacity) and *Km* is a constant with a value equal to the plasma concentration at which the rate of metabolism is one-half the maximum.

As describe in chapter 1, section 1.5.5, *Vmax* and *Km* can be estimated by plotting clearance[$S.F.R/C_{ss}$] against *R(dose/day)*. The point of intercept on the y-axis is the value for *Vmax (mg/day)* while the slope is the negative value for *Km(mg/l)*. Thus, at least two dose-level pairs are needed in plotting the desired graph. The equation of $y = a + bx$ is defined as the regression equation, where *a* is the intercept (equal to *Vmax*) and *b* is the regression coefficient or gradient and is equal to *Km* . Similarly, multivariate regression was also employed to ascertain the relationship of continuous variables with the earlier equation.

5.8 Statistical tests

Data was stratified according to ethnic and age group. Analysis of variance(ANOVA) is used in investigating differences between continous data(age, weight, length of disease years, dose, serum concentration, clearance) in each ethnic groups. Degree of significance is set at $p \leq 0.05$.

The method proposed by Greenland (1971) in estimating trend from the slope(β) of the regression equation was employed in determining difference between groups(eg. ethnic, age). Detailed outline of the formulas used was described in chapter 3, section

3.3.3b Multivariate regression equation was later used for modeling if the relation is suspected to be linear. (Chapter 3, section 3.2.4). Similarly, variances of slope(β) or mean values of parameters(eg. clearances, *Km, Vmax*(constants (α, β)) was calculated by the formulas described by Armitage and Berry (1997) and described in chapter 3. These variances (β, α) were used to calculate the summary estimate, Q statistic (chi square statistic) and correlation values for homogeneity, and the 95% confidence interval and summary estimate of each individual groups. The degree of significance is also set at $p \leq 0.05$. All confidence intervals are 95%.

Part 3

Data analysis and outcome

Chapter 6

Therapeutic Drug Monitoring of antiepileptic drugs

6.1 Introduction:

Therapeutic drug monitoring or clinical pharmacokinetics is defined by the process of using drug concentrations, pharmacokinetic principles, and pharmacodynamic criteria to optimize therapy [Evans 1991]. Optimizing therapy of AED drugs by therapeutic drug monitoring is accomplished by minimizing the probability of toxicity or by increasing the probability of the desired therapeutic effect. The desired therapeutic effect or the occurrences of toxicity are usually associated with a certain concentration range. These concentration ranges are termed the therapeutic range or therapeutic window. Therapeutic range is defined as a range of drug concentration within which the drug is effective and the probability of unacceptable toxicity is low. Currently, the value and reasons for therapeutic drug monitoring have been well established for the aminoglycosides class of antibiotics, digoxin, theophylline and for established antiepileptic drugs(AED) such as phenytoin, carbamazepine, sodium valproate and phenobarbitone [Evans et al 1991].

Other than the phenomena of narrow therapeutic range, these AEDs are also associated with high intra and inter-individual variation between patients[Welty et al 1983]. This then led to the use of these AEDs being individualised to suit to patient's needs. Individualisation in therapy can be best established by proper monitoring or titration of these drugs.

Studies on therapeutic drug monitoring of AEDs [Frewin et al 1982, Larkin et al 1991, Karande et al 1992, Mckee et al 1993, Eadie 1994] had all shown the benefits of antiepileptic drug monitoring for chronic epileptics. The reports of higher number of serum levels in target range, improvement in therapeutic response and reduced hospital visits had indicated the advantage and the critical nature of this service for patient care. However, interpretation of a single measurement of serum level is often difficult and sometimes impractical unless the drug pharmacokinetics and time of sampling are known [Chadwick 1994]. Currently, therapeutic drug monitoring is warranted when certain conditions and disease states led to changes of the pharmacokinetics of these drugs. These include suspected non-compliance, signs of

toxicity, drug-drug interactions, pregnancy and diseases of the kidney and liver [Commission on AEDs, International League Against Epilepsy 1993]. The pharmacokinetic changes are often complicated and monitoring of plasma concentration helps to identify and rectify problems in treatment. These recommendations for monitoring were later upheld by the British Epilepsy Association (1994) and they stressed that therapeutic drug monitoring should function as a tool to improve pharmacotherapy only if clinical judgment failed in its role in the use of these AED in epilepsy.

The above recommendation however prompted two questions pertaining to its validity. The first question is the method used to derive the above recommendations. It should be mentioned that most studies on established AEDs have been noted to be poorly designed [Coatsworth 1971] and systematic reviews to provide evidence towards these recommendations are yet to be published. The second is the problem in applying these recommendation to epileptic patients worldwide when inter-ethnic differences in metabolism have been reviewed for phenytoin [Edeki & Brase 1995] and reported for carbamazepine [Lin et al 1991, Yoon et al 1997]. Inter-ethnic differences have been shown to be significantly important from published European, American or Japanese based studies and extending these recommendations to all populations might be inappropriate.

The current therapeutic ranges of antiepileptic drugs such as phenytoin, carbamazepine, valproic acid and phenobarbitone are well established [Brodie & Dichter 1997]. Studies that showed their importance are plentiful but reports of their use in patients from the Malaysian society were rather limited [Ismail & Rahman 1990, Ismail & Rahman 1993]. These studies came mainly from the predominantly Malay population and failed to mention the therapeutic outcome of treated patients within the desired therapeutic range. Evidence towards the benefits of therapeutic drug monitoring of established antiepileptic drugs in the multi-ethnic society of Malaysia is thus the main objective of this study. The benefits will be measured as the ratio of seizure control between controlled to uncontrolled patients within the specified therapeutic range.

This study will nevertheless have three main objectives. Firstly, to determine the importance of the therapeutic or target range to attain maximum therapeutic

response of established AEDs, secondly, to investigate whether therapeutic response is affected by inter-ethnic differences, and finally, to establish if age is an important determinant for optimum therapeutic response.

6.2 Methodology

The total of 874 and 341 adult and paediatric patients were selected. Patients were selected only if they were on antiepileptic drug therapy for at least a month and were confirmed epileptics with no known metabolic or other neurological disorder. They were checked for non-compliance to the prescribed drug(s) based on the written notes or comments made in the therapeutic request forms. Patients were grouped in their respective ethnic background. No age limit was set and patients were categorised as adults or children. The age range for children is set from zero to less than 18 years while patients age higher than the set limit(18 years old) are referred as adults.

Table 6-1 and 6-4 highlighted the demographic and clinical data of paediatric and adult epileptic patients of each individual hospital visited. Patients were categorised into epilepsy types namely generalised(code 1), partial(code 2) or others(code 3). Epilepsy categorised as others include specific epileptic syndromes and unclassified epilepsies. Examples of specific epileptic syndromes are febrile convulsions, reflex epilepsies and seizure induced seizures while unclassified epilepsies are neonatal seizures and nocturnal tonic-clonic seizures.

To evaluate the effectiveness of these documented therapeutic ranges of the four studied drugs on the control of seizures, the Mantel-Haenszel fixed effect model method for pooling across data was employed. This method basically measures the ratio of effect on two different treatment, typically known as odds ratio. This involved arrangement of data into 2 X 2 table as described in chapter 3. This provides a mathematical relationship of the effect estimate with the summary estimate or the pooled estimate. Radhakrishna 1965 also reported that Mantel-Haenszel chi-square has good statistical properties and is a powerful test. To compare heterogeneity across groups, the chi-square statistic [Mantel 1963], variance of the summary effect measure [Robins, Greenland, Breslow 1986], and a test for homogeneity of effect size across data [Mantel, Brown, Byar 1977] were also used.

Table 6-1 : Statistical biodata for paediatric and adult patients on phenytoin therapy

Variables	Johor Bahru	Permai, Johor Bahru	Klang	Ipoh	Penang
1. *Children*					
No. of patients	47	1	18	4	9
Age(years, sd), range	12.70(4.41), 3-17	17	14.69(4.04),5-17	14.62(1.38),13-15.5	14.56(3.17),12-16
Weight(kg,sd)	37.56,14.96	59.00	42.76,15.78	45.50,5.92	43.78,21.03
Disease duration(years,sd)	5.35,4.12	0.25	6.99,4.08	12.25,4.27	8.00,3.84
Epilepsy types(1:2:3)	25:4:18	1	12:5:1	0:4:0	6:3::0
Gender(male:female)	31:16	1:0	10:8	2:2	3:6
Race (Malay:Chinese:Indian)	15:24:8	0:0:1	4:4:10	0:4:0	1:2:6
Therapy;					
Daily dose(mg/day,sd)	239.21,98.66	-	231.00,105.30	315.00,55.30	301.00,55.15
Dose range(mg/day)	15-400	-	45-300	260-400	200-400
No.of measurement(mean per patient)	2.09	-	2.00	2.00	2.67
Mean concentration(mg/L,sd)	13.33,9.61	-	12.35,8.89	13.81,6.42	21.75,12.01
1. *Adult*					
No. of patients	191	78	118	49	170
Age(years, sd), range	33.82(9.99),19-63	32.57(11.36),20-65	37.49(12.02),22-73	32.57(11.36),20-57	38.25(13.28),18-65
Weight(kg,sd)	60.59,11.17	60.74,10.30	56.22,10.81	60.84,10.39	59.09,11.50
Disease duration(years,sd)	12.84,9.85	8.87,8.85	14.47,11.18	11.51,6.96	18.34,12.73
Epilepsy types(1:2:3)	121:34:36	73:5:0	100:16:2	46:3:0	119:43:8
Gender(male:female)	111:80	43:35	58:60	29:20	90:80
Race (Malay:Chinese:Indian)	99:51:41	32:31:15	38:33:47	24:14:12	47:92:31
Therapy;					
Daily dose(mg/day,sd)	268.98,80.07	273.88,74.87	267.92,69.39	297.50,84.24	279.34,72.93
Dose range(mg/day)	100-600	100-600	100-400	100-600	100-460
No.of measurement(mean per patient)	2.21	2.82	2.14	2.19	2.58
Mean concentration(mg/L,sd)	13.42,8.24	13.31,7.43	13.88,9.36	13.31,7.43	15.92,9.85

Table 6-2 : Statistical biodata for paediatric and adult patients on carbamazepine therapy

Variables	Johor Bahru	Permai, Johor Bahru	Klang	Ipoh	Penang
1. *Children*					
No. of patients	54	-	35	18	33
Age(years, sd), range	10.86(4.01), 3-17	-	10.85(5.12), 2-17	12.18(3.68), 6-16.25	12.54(4.00),5-17
Weight(kg,sd)	32.89,14.26	-	31.02,13.44	34.09,12.45	37.06,19.90
Disease duration(years,sd)	5.57,3.63	-	5.45,4.30	8.89,3.86	6.73,4.71
Epilepsy types(1:2:3)	28:10:16	-	26:7:2	16:2:0	19:12:2
Gender(male:female)	20:34	-	15:20	12:6	14:19
Race (Malay:Chinese:Indian)	26:22:6	-	17:0:18	10:2:6	8:19:6
Therapy;					
Daily dose(mg/day,sd)	4.09.40,229.70	-	415.50,236.80	706.20,239.50	500.00,227.60
Dose range(mg/day)	90-900	-	30-900	300-1200	150-1000
No.of measurement(mean per patient)	1.63	-	1.46	1.88	1.79
Mean concentration(mg/L,sd)	3.32,2.00	-	6.82,2.08	6.97,1.55	5.74,1.97
1. *Adult*					
No. of patients	82	56	91	39	131
Age(years, sd), range	33.80(9.47), 18-55	39.45(10.24),18-63	34.04(10.95), 18-59	31.38(10.30), 19-58	33.82(11.48),18.72
Weight(kg,sd)	59.76,10.97	61.33,8.35	53.45,11.04	55.85,10.27	58.05,12.41
Disease duration(years,sd)	12.25,9.26	10.14,8.72	15.24,12.07	16.53,11.23	14.97,11.04
Epilepsy types(1:2:3)	51:15:16	53:3:0	81:10:0	38:1:0	87:38:6
Gender(male:female)	43:39	34:23	35:56	13:26	66:65
Race (Malay:Chinese:Indian)	40:23:19	19:27:11	24:28:39	10:5:24	37:79:15
Therapy;					
Daily dose(mg/day,sd)	522.30,298.60	686.30,218.10	698.60,273.90	758.20,313.90	816.00,346.80
Dose range(mg/day)	100-1500	200-1200	200-1200	200-1500	100-2000
No.of measurement(mean per patient)	1.56	1.31	1.52	1.68	1.82
Mean concentration(mg/L,sd)	5.39,2.54	6.47,1.54	6.69,1.72	7.27,1.80	7.30,2.63

Table 6-3 : Statistical biodata for paediatric and adult patients on valproic acid therapy

Variables	Johor Bahru	Permai, Johor Bahru	Klang	Ipoh	Penang
1. *Children*					
No. of patients	101	-	73	37	33
Age(years, sd), range	9.16(4.35), 18-57	-	9.04(4.24), 5-16	7.79(4.31),1-16.25	10.91(3.74),2-17
Weight(kg,sd)	27.98,13.41	-	28.20,13.14	24.38,11.10	33.42,16.36
Disease duration(years,sd)	6.22,3.78	-	4.58,3.41	7.71,3.94	6.11,3.06
Epilepsy types(1:2:3)	52:12:37	-	51:18:4	31:6:0	24:8:1
Gender(male:female)	52:49	-	1:32	20:17	22:11
Race (Malay:Chinese:Indian)	52:38:10	-	18:4:51	23:4:10	11:16:6
Therapy;					
Daily dose(mg/day,sd)	554.70,344.00	-	477.00,229.20	932.30,497.10	608.50,353.30
Dose range(mg/day)	100-1500	-	90-1000	300-2000	100-1800
No.of measurement(mean per patient)	2.19	-	1.42	1.97	2.46
Mean concentration(mg/L,sd)	63.78,28.61	-	57.54,33.17	76.15,28.62	57.52,31.52
1. *Adult*					
No. of patients	85	26	57	21	62
Age(years, sd), range	31.54(10.58), 18-57	40.04(16.03),18-81	30.91(10.97),18-50	24.99(5.77),18-37	33.56(9.74),19-58
Weight(kg,sd)	57.67,11.31	60.94,9.29	56.21,11.40	52.86,8.43	54.77,8.76
Disease duration(years,sd)	12.23,8.71	12.62,11.96	15.44,9.44	13.12,11.44	14.29,9.75
Epilepsy types(1:2:3)	53:11:21	24:2:0	56:1	17:4:0	42:17:3
Gender(male:female)	43:42	18:8	31:42	4:17	30:32
Race (Malay:Chinese:Indian)	48:17:20	12:6:8	7:13:37	11:10:10	14:44:4
Therapy;					
Daily dose(mg/day,sd)	738.90,362.80	954.50,406.60	792.50,336.30	1247.60,378.20	1000.80,499.30
Dose range(mg/day)	200-1500	200-1800	300-1800	600-2000	200-2600
No.of measurement(mean per patient)	1.80	1.69	1.63	2.00	1.98
Mean concentration(mg/L,sd)	41.32,19.35	37.56,16.25	48.34,29.80	58.07,21.03	41.74,22.98

Table 6-4 : Statistical biodata for paediatric and adult patients on phenobarbitone therapy

Variables	Johor Bahru	Permai, Johor Bahru	Klang	Ipoh	Penang
1. *Children*					
No. of patients	39	-	16	-	12
Age(years, sd),range	8.10(4.15),2-17	-	5.03(3.62),5-14	-	11.42(3.14),6-17
Weight(kg,sd)	23.52,13.17	-	17.40,10.16	-	28.58,10.81
Disease duration(years,sd)	4.62,3.28	-	3.54,2.07	-	7.42,3.88
Epilepsy types(1:2:3)	22:2:15	-	13:2:1	-	10:2:0
Gender(male:female)	30:9	-	12:4	-	8:4
Race (Malay:Chinese:Indian)	24:11:4	-	8:2:6	-	2:6:4
Therapy;					
Daily dose(mg/day,sd)	67.46,38.80	-	53.53,19.82	-	65.00,24.42
Dose range(mg/day)	20-210	-	25-900	-	20-105
No.of measurement(mean per patient)	1.80	-	1.06	-	1.17
Mean concentration (mg/L,sd)	14.78,9.69	-	10.34,14.06	-	19.26,13.96
1. *Adult*					
No. of patients	31	90	10	6	49
Age(years, sd), range	30.94(8.42),20-35	43.19(12.64), 18-91	34.00(4.69),24-40	23.96(4.78),12-30	37.22(9.22),22-53
Weight(kg,sd)	58.65,11.61	61.24,8.68	60.50,7.20	57.57,4.41	61.02,11.23
Disease duration(years,sd)	13.58,7.87	12.84,9.08	12.00,4.57	13.00,7.07	20.14,12.79
Epilepsy types(1:2:3)	22:2:7	85:5:0	6:4:0	6:0:0	31:15:13
Gender(male:female)	17:14	48:42	2:8	2:4	34:15
Race (Malay:Chinese:Indian)	11:12:8	31:39:20	4:4:2	4:0:2	17:38:4
Therapy;					
Daily dose(mg/day,sd)	75.47,49.29	107.05-54.52	60.25,58.00	97.50,52.50	73.40,39.50
Dose range(mg/day)	30-270	15-330	30-90	30-180	15-180
No.of measurement(mean per patient)	1.71	1.64	1.20	2.00	1.56
Mean concentration(mg/L,sd)	14.02,7.60	18.80,11.31	9.50,4.30	25.99,9.06	13.71,7.42

Since most patients were on two or more different dosage regimens, only one dose and serum level pair and the corresponding treatment response from each patient was randomly taken. Treatment response was defined as the number of seizures recorded by patients and were broadly classified either controlled or non-controlled. Records for seizures were obtained from patients' record book where seizures were noted down by each patient's carers. Record for seizures for hospitalised patients were gathered from seizure charts. Randomisation was based on computer selected set of numbers for each dose-serum level pair(Appendix 6-1). This method of randomisation was used as to avoid bias in selection all dose-serum level pairs.

No attempts were made to separate monotherapy and polytherapy patients since this study was aimed to examine the effectiveness of the defined therapeutic ranges of phenytoin(10-20mg/L), carbamazepine(4-12mg/L), valproic acid(50-100mg/L) and phenobarbitone(15-40mg/L). The hypothesis of this study is thus to test the difference in odds ratio(ψ) of controlled and uncontrolled epileptic patients in the so-called therapeutic ranges between Malay(ψ_m), Chinese(ψ_c) and Indian(ψ_i) patients, for example, H_0 ; $\psi_m = \psi_c$ and H_1 ; $\psi_c \# \psi_m$. ψ_m denotes odds ratio of Malay epileptic patients while ψ_c is odds ratio of Chinese epileptic patients.

ANOVA one way analysis of variances and t-test were used for comparison of age, weight, disease years and dose per kilogram among ethnic groups. P value equal or less than 0.05 was considered significant in determining homogeneity, difference in odds ratio and ANOVA one way analysis of variances. The parameter used in the analysis were the mean dose per kilogram and serum levels in which levels were expressed in mg/L.

6.3 Results

6.3.1 Phenytoin

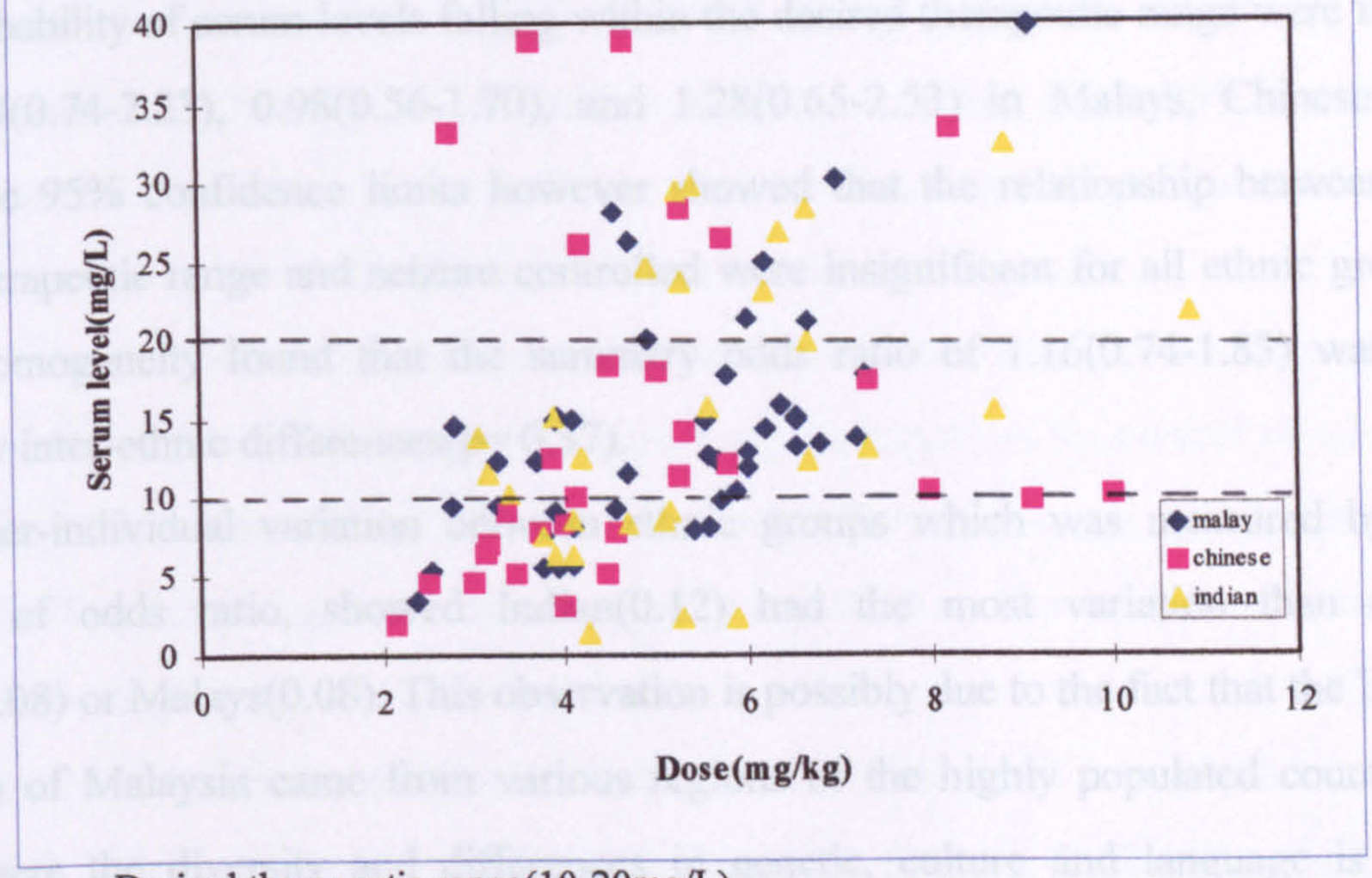
Table 6-5 details clinical data and odds ratio of patients on phenytoin either on monotherapy or polytherapy. Test of one-way analysis of variance(ANOVA) on age($p = 0.08$), weight($p = 0.07$) and disease years($p = 0.27$) showed no significant difference was found to exist on the population sample of Malay, Chinese and Indian patients. Mean doses($p = 0.42$) and serum levels($p = 0.06$) were found to be similar for all three ethnic groups.

Table 6-5 : Clinical data and odds-ratios of patients on phenytoin therapeutic range of 10-20mg/L.

Variables	Malay			Chinese			Indian		
	Controlled	All	Not-controlled	Controlled	All	Not-controlled	Controlled	All	Not-controlled
I. Children/Adult	90		170	82		173	61		110
-age(years,sd)		34.55,13.19			34.10,14.54			31.73,11.93	
-weight(kg,sd)		58.26,12.49			56.46,13.30			55.40,13.34	
-disease years(years,sd)		12.40,10.52			13.95,11.57			13.05,10.03	
-dose(mg.kg,sd)	5.18,1.79		5.12,1.82	4.78,2.50		5.30,1.85	5.36,2.08		5.54,2.39
-dose-range	0.18-10.26		0.18-12.50	0.17-15.00		0.20-12.00	0.33-12.50		0.08-14.29
-serum level(mg/L,sd)	15.85,9.20		16.43,9.24	17.11,11.40		18.65,9.53	15.68,9.78		19.28,11.34
-serum level range	2.84-39.90		2.11-41.29	1.85-39.43		1.85-42.65	1.00-41.72		3.88-47.20
-ratio In/Out TR	47:43		78:92	29:53		62:111	24:37		37:73
-odds-ratio		1.29			0.98			1.28	
95%, C.I		0.74-2.23			0.56-1.70			0.65-2.53	
-variance		0.08			0.08			0.12	
Pooled odds-ratio					1.16				
-95%, C.I					0.74-1.83				
-p					0.37				

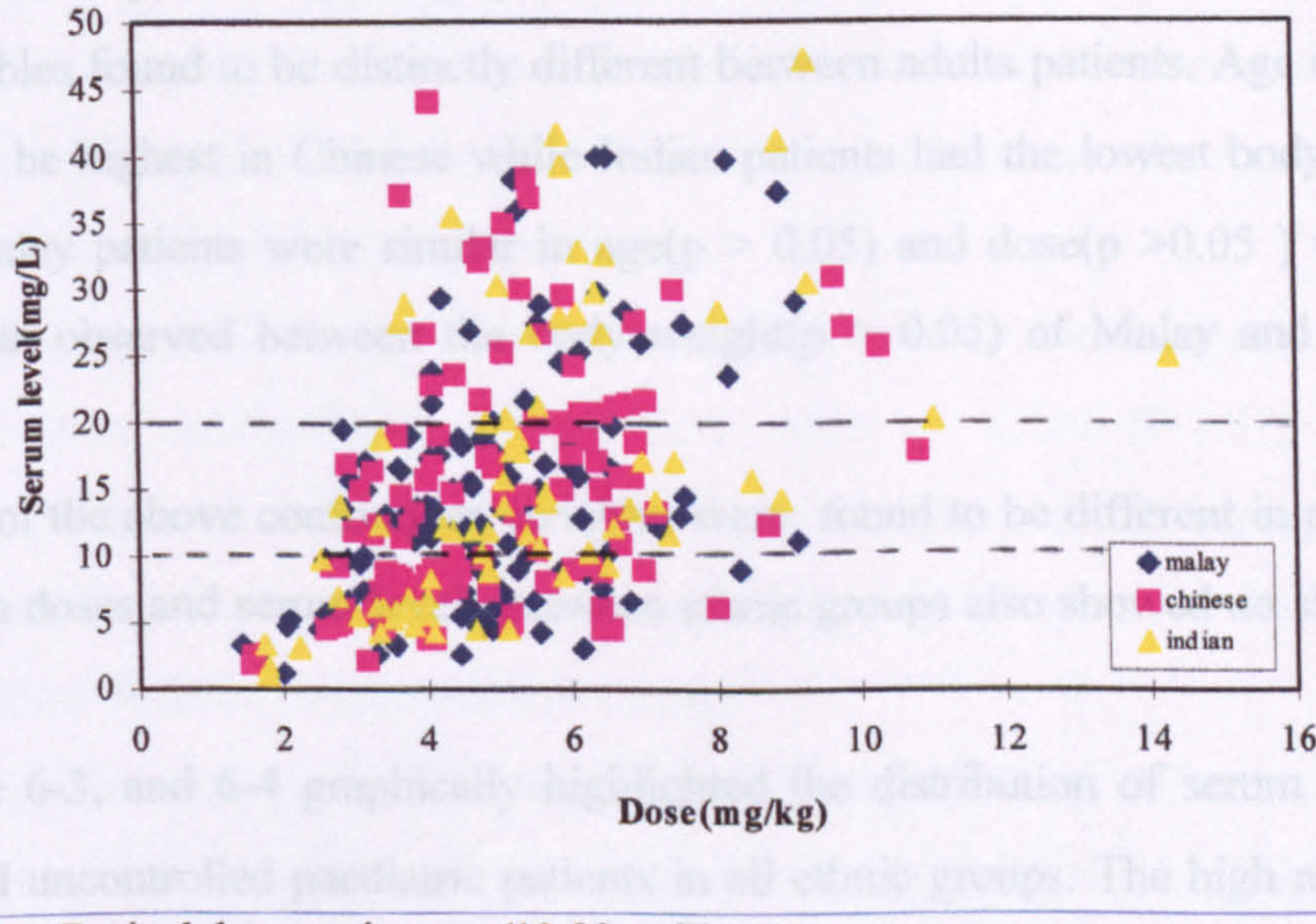
Abbreviation: In/Out TR, inside or outside therapeutic range

Figure 6-1: Phenytoin dose and serum level in controlled epileptic patients



----- : Desired therapeutic range(10-20mg/L)

Figure 6-2: Phenytoin dose and serum level in uncontrolled epileptic patients



------ : Desired therapeutic range(10-20mg/L)

Figure 6-1 and 6-2 presented the distribution of doses and serum levels in controlled and uncontrolled patients of the three ethnic groups respectively. Serum levels of controlled patients were well scattered with only 42.92% falling within the desired therapeutic range as compared to 39.07% of uncontrolled patients. Odds ratio(95%, C.I) for the probability of serum levels falling within the desired therapeutic range were found to be 1.29(0.74-2.23), 0.98(0.56-1.70), and 1.28(0.65-2.53) in Malays, Chinese and Indian. The 95% confidence limits however showed that the relationship between the desired therapeutic range and seizure controlled were insignificant for all ethnic groups. Test of homogeneity found that the summary odds ratio of 1.16(0.74-1.83) was not effected by inter-ethnic differences($p = 0.37$).

Inter-individual variation between ethnic groups which was measured by the variances of odds ratio, showed Indian(0.12) had the most variation than either Chinese(0.08) or Malays(0.08). This observation is possibly due to the fact that the Indian population of Malaysia came from various regions of the highly populated country of India where the diversity and differences in genetic, culture and language is most prominent.

6.3.2 Carbamazepine

Table 6-6 presented the results of paediatric and adult patients of different ethnic groups on carbamazepine therapy. Age($p = 0.003$), weight($p = 0.003$) and dose($p = 0.008$) were the variables found to be distinctly different between adults patients. Age and dose were found to be highest in Chinese while Indian patients had the lowest body weight. Indian and Malay patients were similar in age($p > 0.05$) and dose($p > 0.05$) while no differences was observed between the body weight($p > 0.05$) of Malay and Chinese patients.

None of the above confounder variables were found to be different in paediatric patients. Mean doses and serum levels between ethnic groups also showed no significant differences.

Figure 6-3, and 6-4 graphically highlighted the distribution of serum levels of controlled and uncontrolled paediatric patients in all ethnic groups. The high number of serum levels within the desired therapeutic range of 4-12mg/L in both controlled and

uncontrolled patients demonstrated the lack of significant relationship between serum level and therapeutic outcome.

Table 6-6 showed that the odds ratio of controlled to uncontrolled patients is highest for Malay paediatric patients. This clearly signifies that serum levels of controlled Malay patients showed the highest probability of falling within the therapeutic range when compared to Chinese or Indian patients. Homogeneity test of the 95% confidence limit of these observed odds ratio nevertheless showed there was no significant difference between ethnic groups (p=0.40) although Malays had the widest range.

The lower confidence limit of the odds ratio being less than 1 in all ethnic groups further proved that therapeutic range was statistically unimportant for control of seizures. These findings also demonstrated that the observed differences in age, weight and dose between the three ethnic groups had no significant effect on the pooled odds ratio of efficacy for the target range of carbamazepine.

Figure 6-5 and figure 6-6 presented the pattern of observed serum levels between ethnic groups in both controlled and uncontrolled adult patients. Odds ratio of all three ethnic groups showed values higher than 1 but their 95% confidence limits showed that these findings were not conclusive for the desired range of 4-12mg/L. Pooled odds ratio(95%, C.I) of 1.58(0.59-5.23) subsequently confirmed these findings(p=0.25).

Inter-patient variability among ethnic groups is measured by their variances. Variances of Malays, Chinese and Indians paediatric patients were found to be between 0.39 and 0.42 These clearly showed that high inter-individual variation between patients do exist in all ethnic groups. The inter-individual differences between ethnic groups were however very small and possibly insignificant.

Test of homogeneity for the odds ratio between paediatric and adult patients in all ethnic groups were insignificant. Pooled odds ratio of 1.07(0.40-2.87) and the corresponding p value of 0.13 clearly demonstrated that the use of target range to ascertain the best therapeutic response is inaccurate and could be related to the high inter and intraindividual differences between individuals.

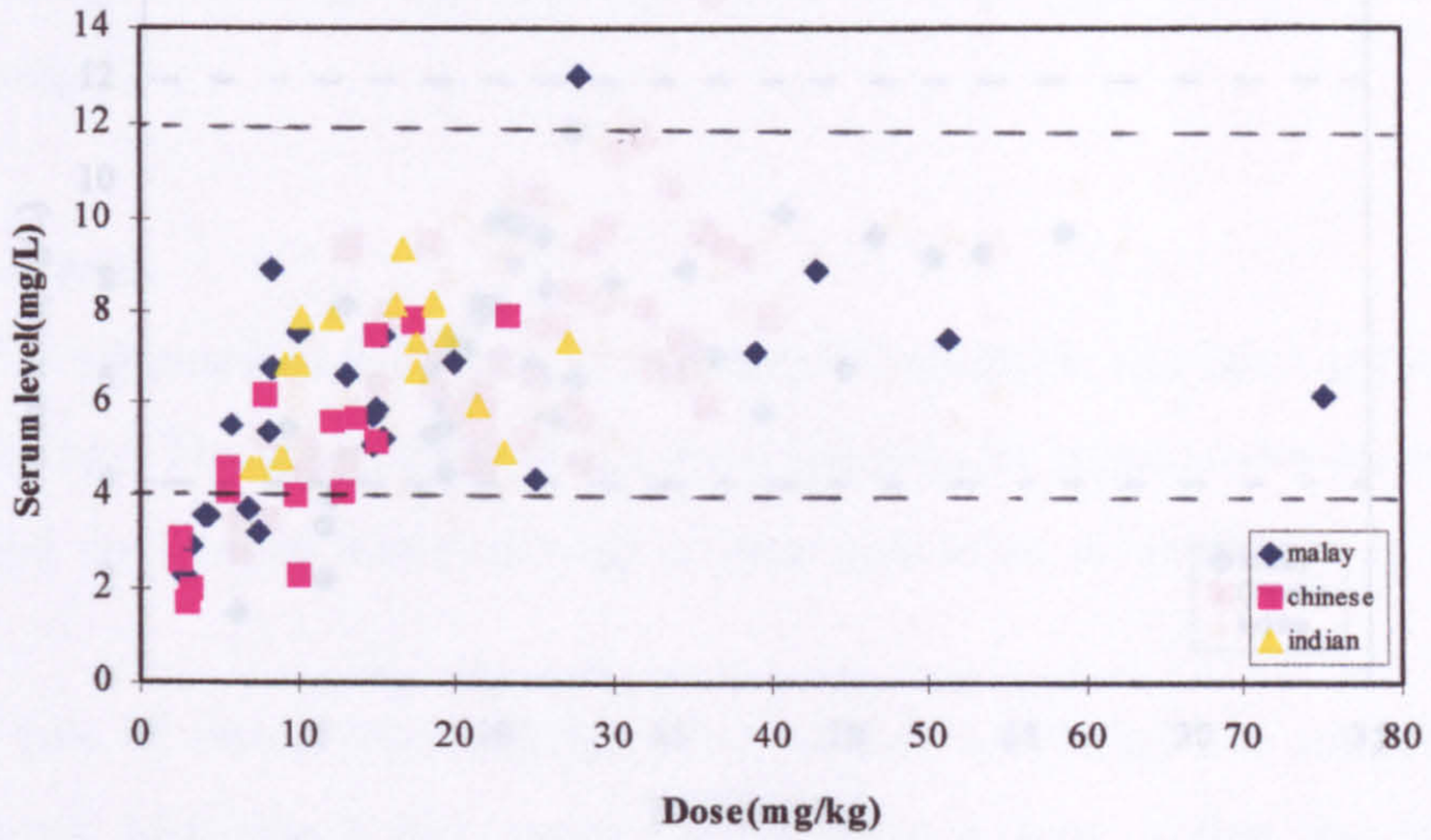
Variances for adult patients showed that large inter-individual differences do exist in all ethnic groups. Inter-individual differences were however highest in Chinese(1.00), Indians(0.99), and Malay(0.42). These findings can be demonstrated by the wide range of odds ratio of each ethnic groups. The differences in adults were more pronounced where

Table 6-6 : Clinical data and odds-ratios of patients on carbamazepine therapeutic range of 4-12mg/L.

Variables	Malay			Chinese			Indian		
	Controlled	All	Not-controlled	Controlled	All	Not-controlled	Controlled	All	Not-controlled
I. Children	24		38	17		26	17		17
-age(years,sd)		10.61,4.57			11.52,4.21			12.32,3.37	
-weight(kg,sd)		30.67,12.13			37.15,14.77			35.71,11.65	
-disease years(years,sd)		6.57,4.50			5.13,3.65			6.21,3.15	
-dose(mg.kg,sd)	18.28,17.70		14.06,8.10	10.43,6.19		17.65,8.69	15.42,5.95		17.13,7.22
-dose-range	2.86-75.00		4.26-36.00	2.50-23.33		4.00-40.00	7.02-27.27		7.14-33.33
-serum level(mg/L,sd)	5.98,2.35		5.76,2.05	4.83,2.11		5.98,2.04	6.86,1.42		6.54-2.24
-serum level range	2.33-13.02		1.98-9.87	1.71-7.91		2.07-10.05	4.60-9.34		2.35-10.70
-ratio In/Out TR	19:5		30:8	12:5		22:4	12:5		16:1
-odds-ratio		1.01			0.44			0.53	
95%, C.I		0.29-3.56			0.13-1.49			0.52-6.75	
-variance		0.41			0.39			0.43	
Pooled odds-ratio					0.66				
-95%, C.I					0.22-2.01				
-p					0.40				
I. Adult	40		90	51		124	51		64
-age(years,sd)		33.21,9.69			36.85,12.25			33.09,10.48	
-weight(kg,sd)		58.23,11.63			58.98,10.82			54.59,10.84	
-disease years(years,sd)		13.38,10.49			14.54,12.14			14.23,10.65	
-dose(mg.kg,sd)	11.58,5.51		10.66,6.63	18.62,4.10		13.65,6.32	13.31,7.55		13.14,5.48
-dose-range	2.56-26.32		1.25-32.50	2.70-8.16		2.53-32.00	3.23-34.88		3.95-26.09
-serum level(mg/L,sd)	6.69,2.13		6.63,2.20	6.60,2.21		6.64,1.95	7.16,1.94		6.30,1.81
-serum level range	1.42-10.99		1.45-12.24	2.63-4.89		2.60-11.12	3.43-11.25		3.00-10.79
-ratio In/Out TR	36:4		78:12	48:3		58:8	49:2		58:6
-odds-ratio		1.38			2.25			2.20	
95%, C.I		0.39-4.85			0.32-15.96			0.31-15.49	
-variance		0.42			1.00			0.99	
Pooled odds-ratio					1.77				
-95%, C.I					0.59-5.23				
-p					0.34				

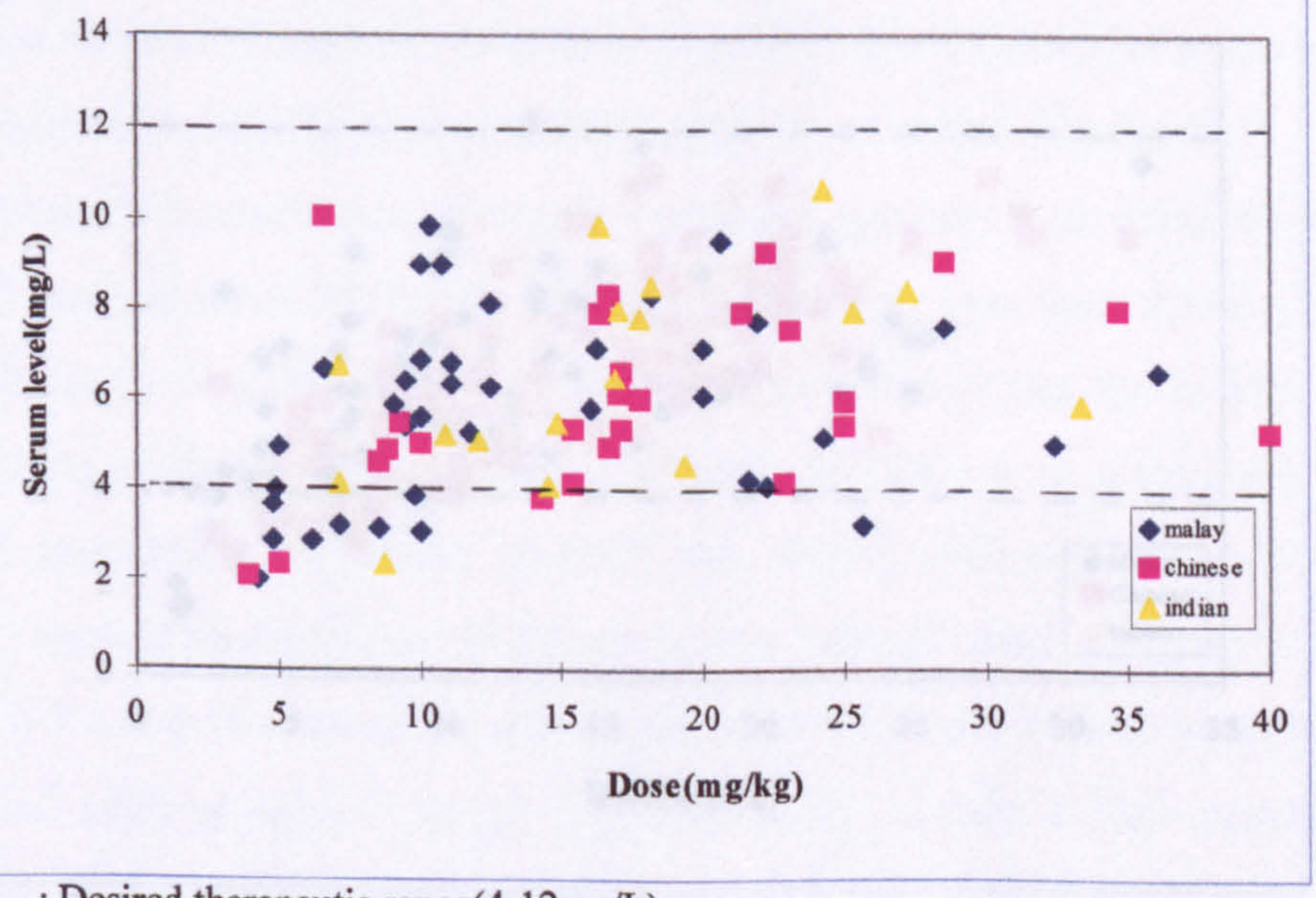
Abbreviation: In/Out TR, inside or outside therapeutic range

Figure 6 - 3 : Carbamazepine dose and serum level in controlled paediatric epileptic patients



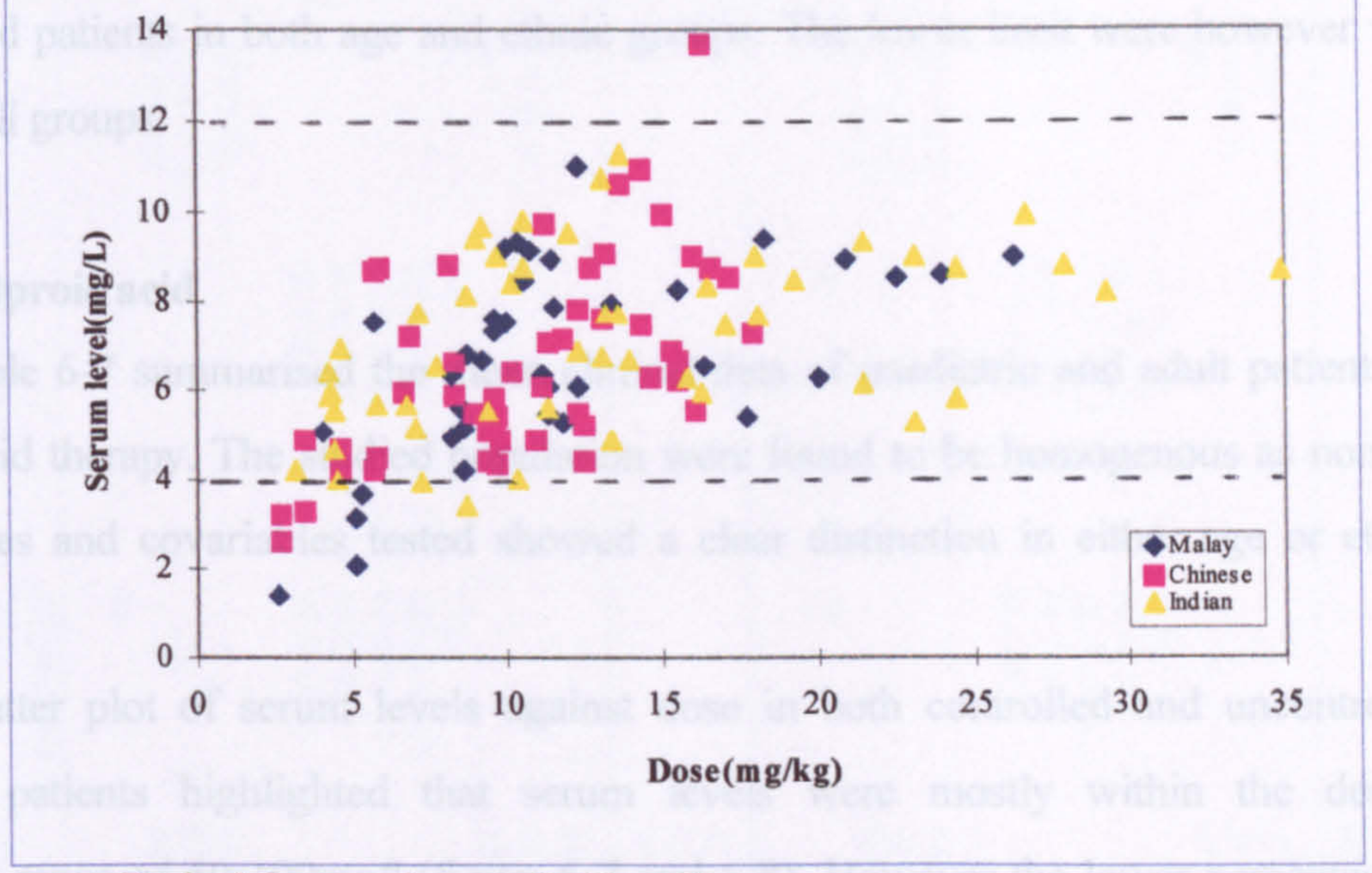
------ : Desired therapeutic range(4-12mg/L)

Figure 6 - 4 :Carbamazepine dose and serum level in uncontrolled paediatric epileptic children



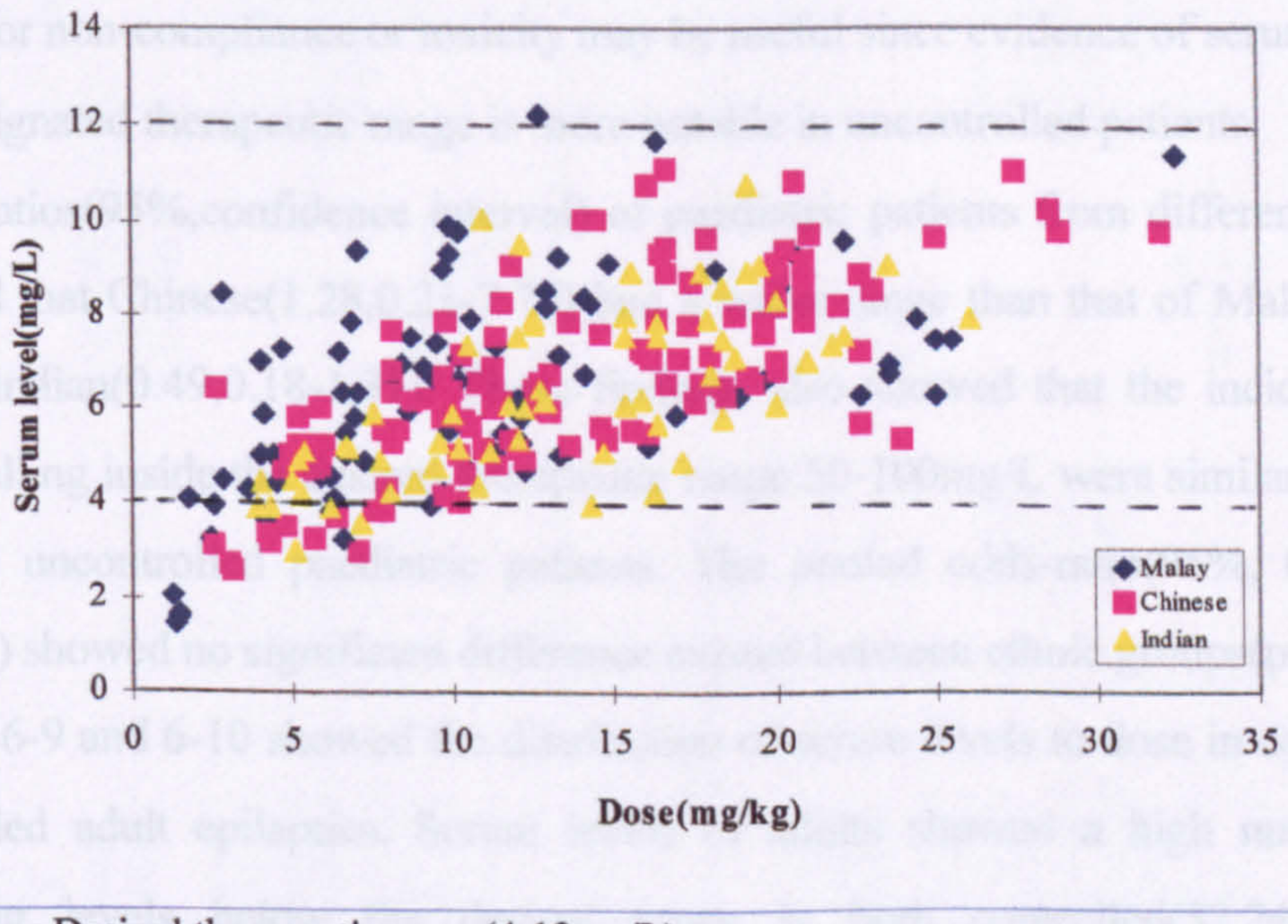
------ : Desired therapeutic renge(4-12mg/L)

Figure 6 - 5 : Carbamazepine dose and serum level in controlled adult epileptic patients



------ : Desired therapeutic range(4-12mg/L)

Figure 6 -6 : Carbamazepine dose and serum level in uncontrolled adult epileptic patients



------ : Desired therapeutic range(4-12mg/L)

the upper confidence limit odds ratio value of 12.06, 10.80 and 4.85 were observed for Indians, Chinese and Malays. However, these observations could also be affected by the differences in sample sizes as the number of controlled patients were smaller than uncontrolled patients in both age and ethnic groups. The lower limit were however very similar in all groups.

6.3.3 Valproic acid

Table 6-7 summarised the mean clinical data of paediatric and adult patients on valproic acid therapy. The studied population were found to be homogenous as none of the variables and covariables tested showed a clear distinction in either age or ethnic groups.

Scatter plot of serum levels against dose in both controlled and uncontrolled paediatric patients highlighted that serum levels were mostly within the desired therapeutic range of 50-100mg/L(figure 6-7 and 6-8). However the lower percentage of serum levels within the therapeutic range for controlled(69.74%) patients than uncontrolled(79.04%) patients showed that determining of serum levels for optimising therapeutic efficacy was still questionable.

The percentage of serum levels outside the target range for uncontrolled and controlled patients were 26.52% and this showed that the determination of serum concentration for non-compliance or toxicity may be useful since evidence of serum levels outside the designated therapeutic range is more notable in uncontrolled patients.

Odds ratios(95%,confidence interval) of paediatric patients from different ethnic groups showed that Chinese(1.28,0.21-7.77) had a wider range than that of Malay(0.55, 0.25-1.18) or Indian(0.49,0.18-1.31). These findings also showed that the incidence of serum levels falling inside the desired therapeutic range 50-100mg/L were similar in both controlled and uncontrolled paediatric patients. The pooled odds-ratio(95%, C.I) of 0.60(0.30-1.22) showed no significant difference existed between ethnic groups(p=0.14).

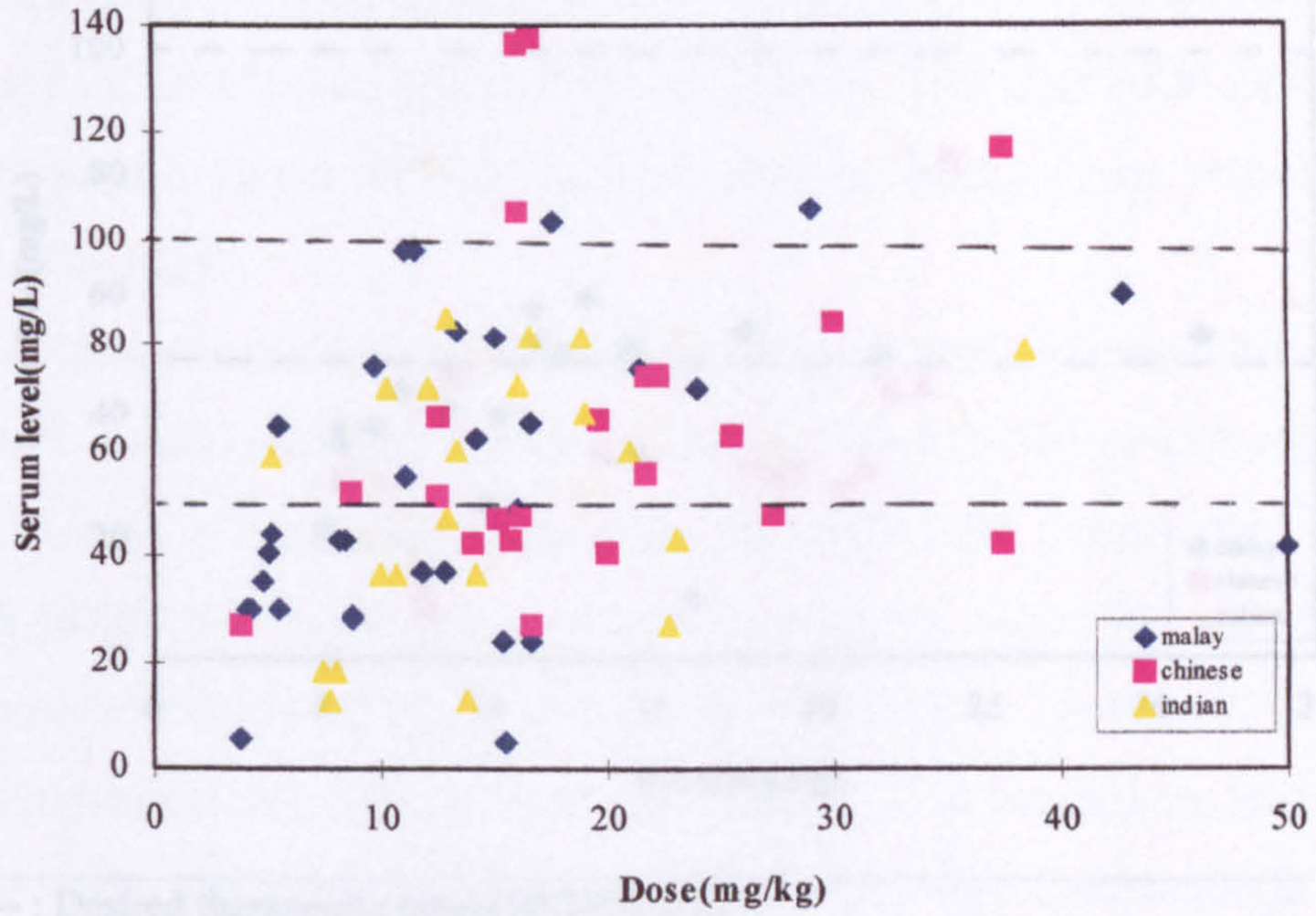
Figure 6-9 and 6-10 showed the distribution of serum levels to dose in controlled and uncontrolled adult epileptics. Serum levels of adults showed a high number of patients having levels below the desired range in both controlled(52.24%) and uncontrolled(55.65%) patient groups. Number of serum levels above the desired therapeutic range were few and only observed in uncontrolled patients. This was expected

Table 6-7 : Clinical data and odds-ratios of patients on valproic acid therapeutic range of 50-100mg/L.

Variables	Malay			Chinese			Indian		
	Controlled	All	Not-controlled	Controlled	All	Not-controlled	Controlled	All	Not-controlled
I. Children	32		84	22		44	21		39
-age(years,sd)		8.65,4.47			8.08,3.98			10.12,4.36	
-weight(kg,sd)		27.22,14.07			26.81,13.19			30.35,12.91	
-disease years(years,sd)		5.63,3.87			6.26,3.91			5.91,3.51	
-dose(mg.kg,sd)	14.01,10.47		27.57,22.19	19.40,8.30		25.87,12.56	14.98,7.38		24.69,13.47
-dose-range	3.77-50.00		5.29-150	3.85-37.50		5.88-62.50	5.26-38.46		6.25-74.07
-serum level(mg/L,sd)	53.25,28.23		60.57,31.07	65.85,32.06		68.51,32.74	51.18,24.52		66.21,34.13
-serum level range	4.51-105.99		4.06-135.35	26.79-137.38		10.69-179.97	12.47-85.00		8.71-143.00
-ratio In/Out TR	20:12			20:26		39:5	13:8		30:9
-odds-ratio		0.55			1.28			0.49	
95%, C.I		0.25-1.18			0.21-7.77			0.18-1.31	
-variance		0.15			0.85			0.25	
Pooled odds-ratio					0.60				
-95%, C.I					0.30-1.22				
-p					0.37				
II. Adult	19		69	14		62	24		49
-age(years,sd)		33.60,11.00			33.00,12.06			30.28,11.19	
-weight(kg,sd)		13.97,9.60			56.54,8.49			55.79,11.62	
-disease years(years,sd)		16.47,9.77			13.96,8.89			13.79,10.89	
-dose(mg.kg,sd)	12.13,6.57		17.66,10.20	15.61,6.79		14.88,7.79	12.50,6.79		15.09,8.69
-dose-range	5.06-31.58		3.33-51.28	5.63-24.00		3.28-41.86	3.77-34.29		3.03-38.46
-serum level(mg/L,sd)	41.88,12.91		43.51,22.09	34.40,17.61		38.81,19.05	39.16-17.41		42.23,17.72
-serum level range	9.99-59.77		3.33-125.34	7.71-82.11		8.47-91.90	15.83-81.99		3.60-74.79
-ratio In/Out TR	10:9		36:33	2:12		29:33	11:13		23:26
-odds-ratio		1.02			0.19			0.96	
95%, C.I		0.37-2.83			0.08-0.47			0.90-2.51	
-variance		0.27			0.22			0.24	
Pooled odds-ratio					0.70				
-95%, C.I					0.30-1.60				
-p					0.20				

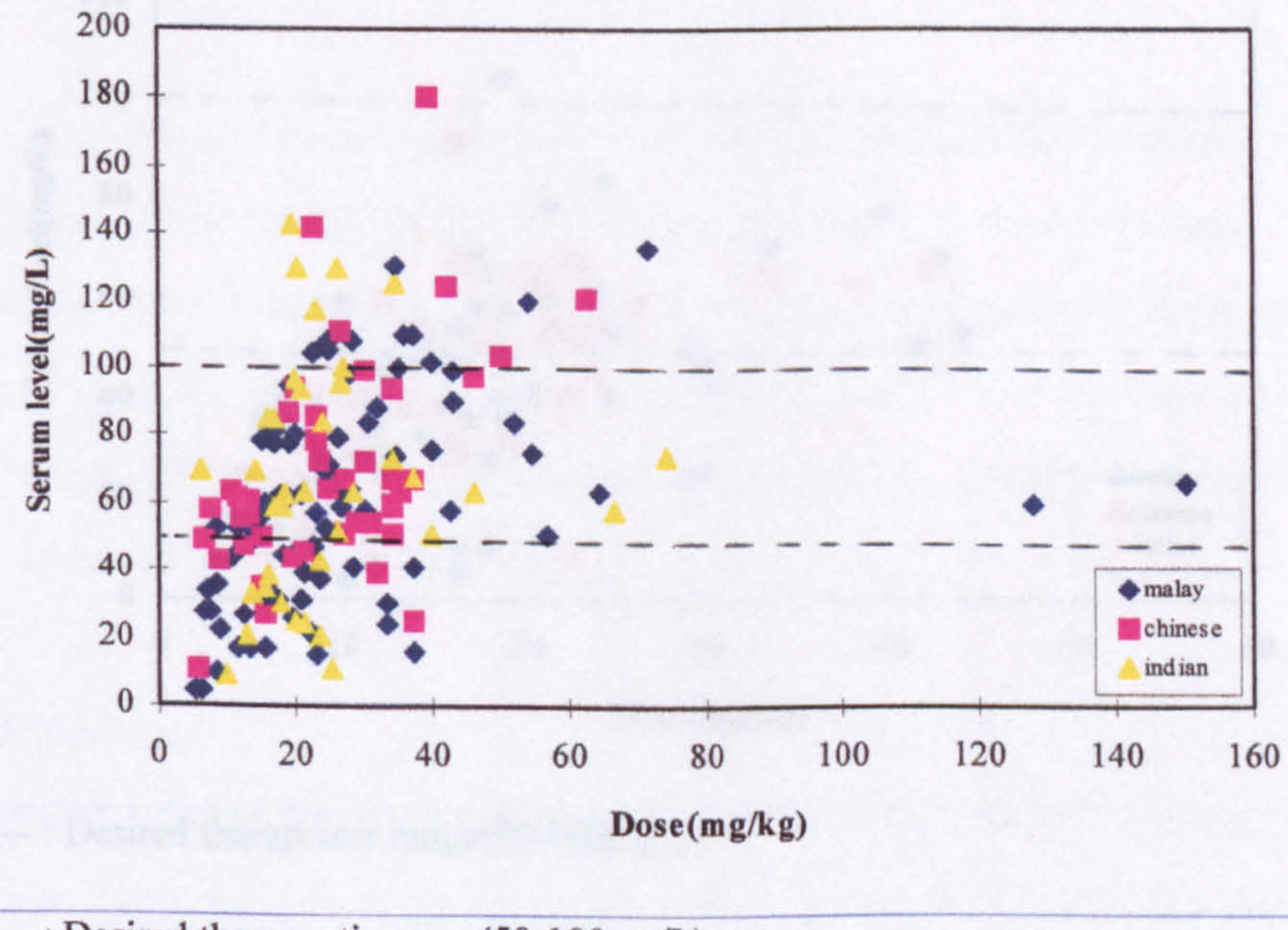
Abbreviation: In/Out TR, inside or outside therapeutic range

Figure 6 - 7 : Valproic acid dose and serum level in controlled paediatric epileptic patients



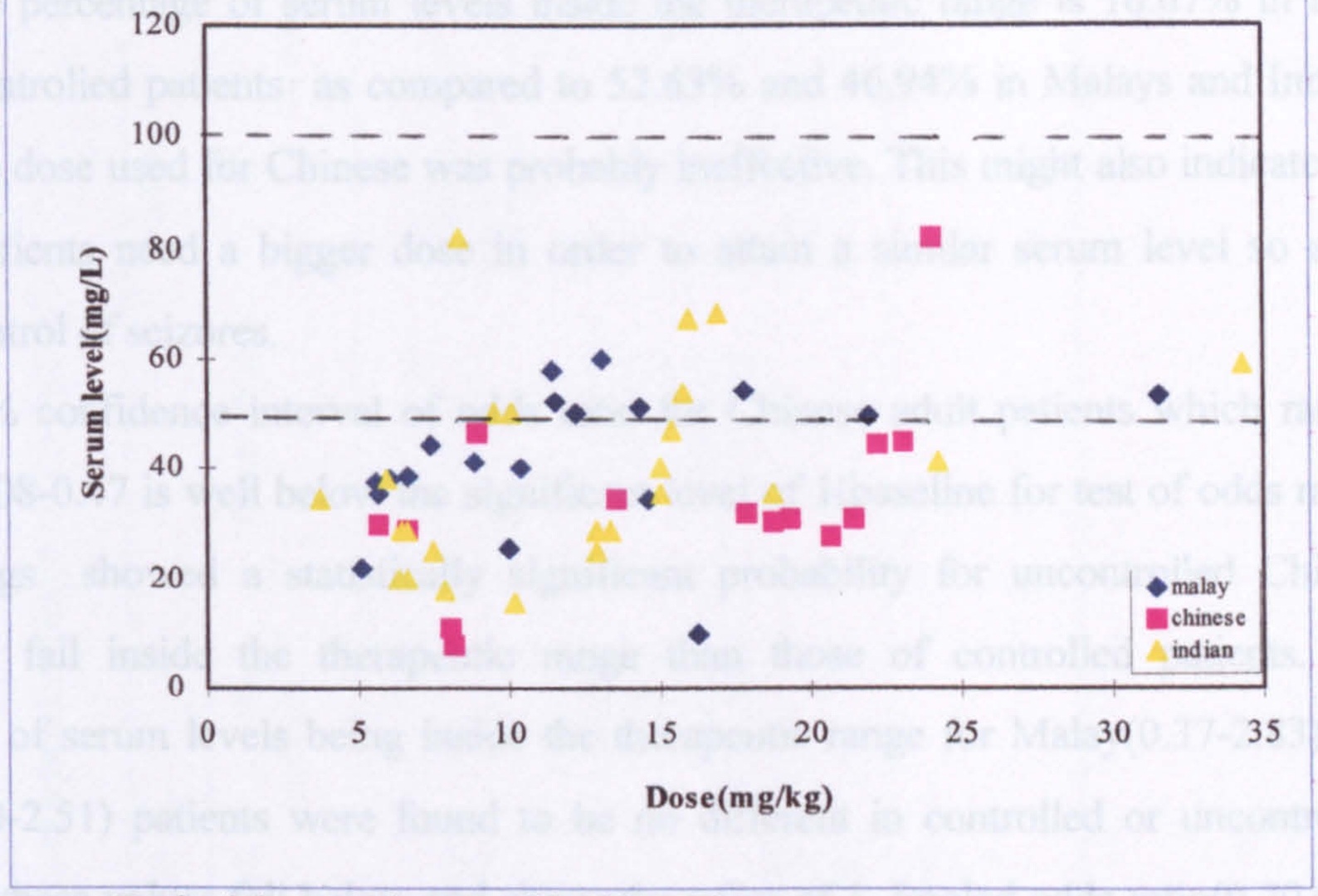
------ : Desired therapeutic range (50-100mg/L)

Figure 6 -8 : Valproic acid dose and serum level in uncontrolled paediatric epileptic patients



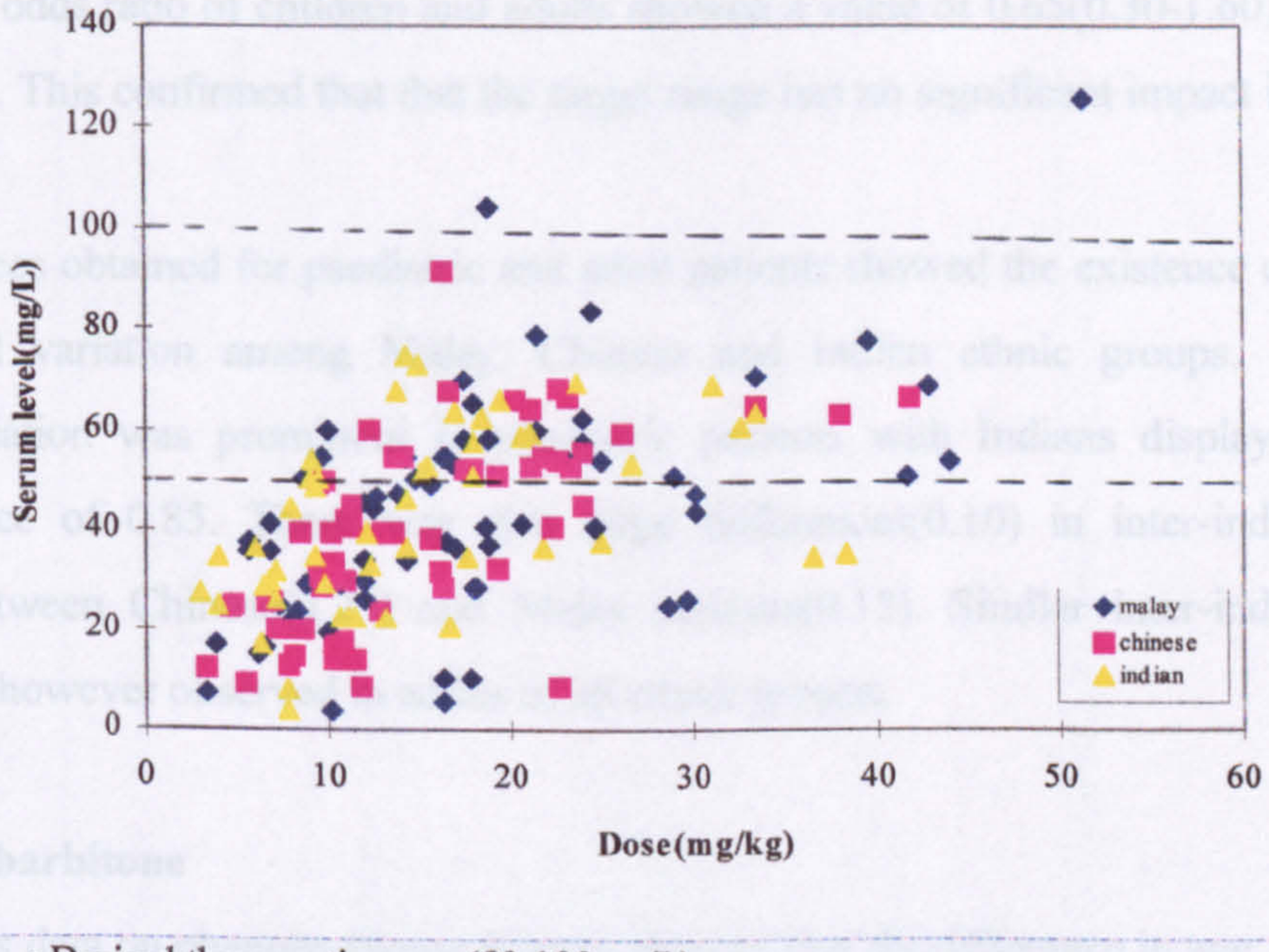
------ : Desired therapeutic range(50-100mg/L)

Figure 6- 9: Valproic acid dose and serum level in controlled adult epileptic patients



------ : Desired therapeutic range(50-100mg/L)

Figure 6-10 : Valproic acid dose and serum level in uncontrolled adult epileptic patients



------ : Desired therapeutic range(50-100mg/L)

since the mean dose used in all ethnic groups were on the lower end of the recommended dose of 20-30mg/kg/day [BNF 1996].

The percentage of serum levels inside the therapeutic range is 16.67% in adult Chinese controlled patients as compared to 52.63% and 46.94% in Malays and Indians indicate the dose used for Chinese was probably ineffective. This might also indicate that Chinese patients need a bigger dose in order to attain a similar serum level so as to achieve control of seizures.

95% confidence interval of odds ratio for Chinese adult patients which ranged between 0.08-0.47 is well below the significant level of 1(baseline for test of odds ratio). The findings showed a statistically significant probability for uncontrolled Chinese patients to fall inside the therapeutic range than those of controlled patients. The probability of serum levels being inside the therapeutic range for Malay(0.37-2.83) and Indian(0.90-2.51) patients were found to be no different in controlled or uncontrolled patients as these values fell below and above the value of 1. Pooled odds-ratio(0.70,0.30-1.60) of the observed differences for three ethnic groups however revealed homogeneity with the degree of significance value of 0.20. This result indicates that the use of therapeutic drug monitoring of valproic acid is highly inconsistent and displayed large inter-patient variation.

Pooled odds ratio of children and adults showed a value of 0.65(0.30-1.60) and a p value of 0.15. This confirmed that that the target range has no significant impact in both age groups.

Variances obtained for paediatric and adult patients showed the existence of high inter-individual variation among Malay, Chinese and Indian ethnic groups. Inter-individual variation was prominent in paediatric patients with Indians displayed the highest variance of 0.85. There was also large differences(0.10) in inter-individual differences between Chinese(0.25) and Malay patients(0.15). Similar inter-individual variation were however observed in adults of all ethnic groups.

6.3.4 Phenobarbitone

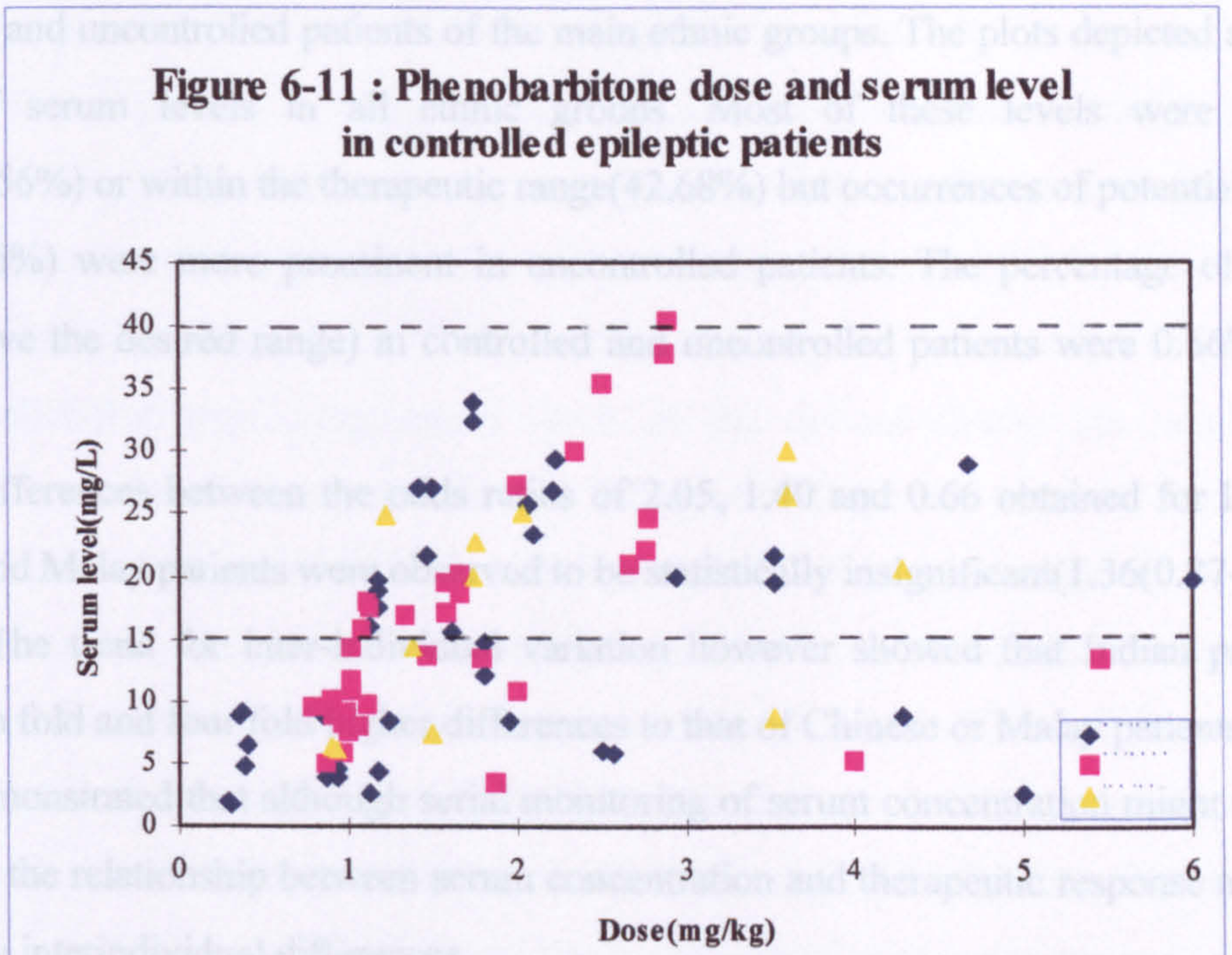
Patients data on phenobarbitone therapy showed that the difference in age, weight and disease years between ethnic groups were insignificant(table 6-8). No significant difference in mean doses and serum levels were noted between ethnic groups.

Table 6-8: Clinical data and odds-ratios of patients on phenobarbitone therapeutic range of 15-40mg/L.

Variables	Malay			Chinese			Indian		
	Controlled	All	Not-controlled	Controlled	All	Not-controlled	Controlled	All	Not-controlled
I. Children/adult	39		57	35		59	12		37
-age(years,sd)		29.27,16.95			33.72,15.12			32.14,19.01	
-weight(kg,sd)		50.34,20.63			54.66,17.35			50.31,19.91	
-disease years(years,sd)		11.95,10.79			12.96,9.70			12.03,9.85	
-dose(mg.kg,sd)	1.86,1.36		1.95,1.37	1.75,1.01		1.62,0.93	2.21,1.20		1.98,1.65
-dose-range	0.29-6.00		0.38-7.83	0.78-5.45		0.24-4.50	0.91-4.29		0.19-6.929
-serum level(mg/L,sd)	14.70,9.71		18.55,11.79	16.02,9.32		13.50,8.28	17.70,8.76		17.97,12.89
-serum level range	1.70-34.01		0.95-48.24	3.55-40.40		1.13-46.70	5.99-30.17		3.37-44.85
-ratio In/Out TR	20:19		35:22	16:19		14:45	7:5		15:22
-odds-ratio		0.66			1.40			2.05	
95%, C.I		0.32-1.36			0.44-4.48			0.42-9.97	
-variance		0.14			0.35			0.65	
Pooled odds-ratio					1.03				
-95%, C.I					0.50-2.10				
-p					0.11				

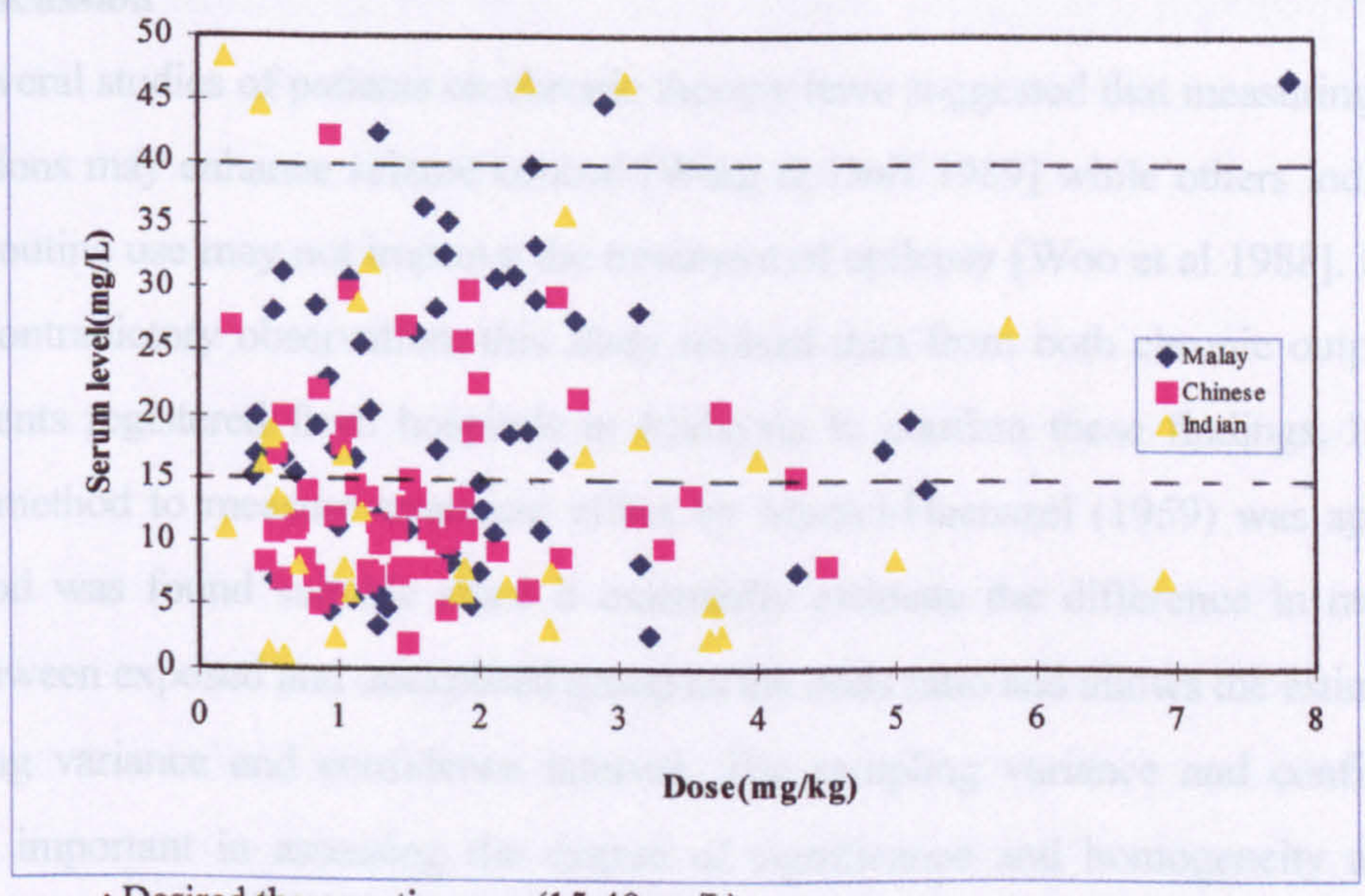
Abbreviation: In/Out TR, inside or outside therapeutic range

Figure 6-11 : Phenobarbitone dose and serum level in controlled epileptic patients



------ : Desired therapeutic range(15-40mg/L)

Figure 6-12 : Phenobarbitone dose and serum level in uncontrolled epileptic patients



------ : Desired therapeutic range(15-40mg/L)

Figure 6-11 and 6-12 presented scatter plot of serum levels and dose for controlled and uncontrolled patients of the main ethnic groups. The plots depicted a wide scatter of serum levels in all ethnic groups. Most of these levels were either below(53.56%) or within the therapeutic range(42.68%) but occurrences of potential toxic levels(3.76%) were more prominent in uncontrolled patients. The percentage of toxic levels(above the desired range) in controlled and uncontrolled patients were 0.66% and 10.34%.

Differences between the odds ratios of 2.05, 1.40 and 0.66 obtained for Indian, Chinese and Malay patients were observed to be statistically insignificant(1.36(0.87-2.14), p=0.11). The trend for inter-individual variation however showed that Indian patients have a two fold and four fold higher differences to that of Chinese or Malay patients. This clearly demonstrated that although serial monitoring of serum concentration might not be important, the relationship between serum concentration and therapeutic response may be affected by interindividual differences.

These results showed that monitoring of phenobarbitone serum levels are unimportant and irrelevant. Its used in the determination of compliance and suspected toxicity may be of clinical importance. These findings showed that the therapeutic range of 15-40mg/L of phenobarbitone had little or no effect in ensuring adequate therapeutic response.

6.4 Discussion

Several studies of patients on chronic therapy have suggested that measuring drug concentrations may enhance seizure control [Wing & Duff 1989] while others indicated that their routine use may not improve the treatment of epilepsy [Woo et al 1988]. Based on these contradictory observation, this study utilised data from both chronic outpatient and inpatients registered from hospitals in Malaysia to confirm these findings. In this study the method to measure treatment effect by Mantel-Haenszel (1959) was applied. The method was found suitable since it essentially estimate the difference in rates of disease between exposed and unexposed group as the odds ratio and allows the estimation of sampling variance and confidence interval. The sampling variance and confidence interval is important in assessing the degree of significance and homogeneity among treatment groups.

In this study, the two treatment patient groups were referred to as controlled(responsive) and uncontrolled(non-responsive) group. The groups were tested on the number of positive responses(no seizures) at a defined therapeutic range of each antiepileptic drug. Odds ratios and the corresponding 95% confidence interval allows significance test for the hypothesis and provide a measure of precision.

Pooled odds ratios of controlled and uncontrolled patients on each of the studied drug revealed that monitoring serum levels at the defined therapeutic range was inadequate and clinical assessment is still the crucial factor for the best therapeutic outcome. These findings were further strengthened by the high incidences of inter-individual variation observed in each ethnic groups. Similar reports of poor correlation by studies between serum levels and seizure control for phenytoin [Dawson & Jamieson 1971], carbamazepine [Callaghan et al 1978, Shorvon et al 1978], phenobarbitone [Buchanan & Allen 1971], and valproic acid[McQueen et al 1982] showed that inter-individual variation is an important factor that restricts the use of target therapeutic range across all individuals. These results thus distinctly validate the current guidelines for monitoring of these established antiepileptic drugs.

The present study was not only aimed to verify the current recommendation but also to examine the impact of inter-ethnic differences to treatment. Odds ratio of Malays, Chinese and Indians epileptic patients on phenytoin, carbamazepine, valproic acid and phenobarbitone therapy revealed that the difference between controlled patients within and outside the defined therapeutic range was statistically insignificant. Results from this multiethnic Malaysian patients although similar to earlier reports[Froescher et al 1981, Beardsley et al 1983, Bussey & Hoffman 1983] had furnished further evidence for control on the seemingly important request for serial serum concentration of these drugs. These findings also showed that without appropriate indication for monitoring, the use of these therapeutic ranges would not be beneficial for optimising therapy[Schoenenberger et al 1995].

High inter-individual variations for all four AEDs had been reported[Schmidt & Haenel 1984, Strandjord & Johannessen 1980, Bruni et al 1978] and had clearly demonstrated that individualised therapy is crucial in therapy. Although similar inter-individual variations for all three ethnic groups were also observed in the present study, the rate of variation for Chinese and Indian patients were more pronounced than Malay

patients. These differences are an indication that the Chinese and Indian population of Malaysia are a diverse group of people and the use of a single population pharmacokinetics for all groups of patients is not appropriate.

Age had not been reported to have any correlation with the variation in response with respect to the desired therapeutic range of carbamazepine [Schmidt & Haenel 1984, Strandjord & Johannessen 1980, Semah et al 1994] and valproic acid [Turnbull et al 1983, Haigh D et al 1975, Jeavons et al 1975]. These findings were similarly demonstrated in this study by the pooled odds ratios(95%,C.I) where the observed differences in controlled and uncontrolled adult and paediatric patients(all ethnic groups) on carbamazepine, 1.07(-0.49 to 1.64) and valproic acid, 0.65(-0.25 to 1.05) had a p value of greater than 0.05. This again showed the universal trend at which the association of seizure control and therapeutic range at different age groups.

Although the Commission on AEDs, International league against epilepsy (1993) had already listed three common pitfalls in therapeutic monitoring as high intra and inter-individual variation on the degree of control to similar serum concentration, discrepancies in sampling time and accuracy in measurement, the above results could also be explained by the following practices. First, although monitoring of antiepileptic drug concentrations can be helpful in optimising therapy, rigorous adhering to the narrow concentration can result to medical practitioners treating blood level and not the patient [Dodson 1989].

Secondly, therapeutic or target range should be regarded as an approximation obtained from population data and restriction to these so-called target range can lead to treatment failure. These practices are definitely inappropriate since studies reporting of patients being well-controlled at serum concentrations below the lower limit [Woo et al 1988] or requiring concentration well above the range to become seizure free [Gannaway et al 1981] had certainly provided proof of the extent to which these target ranges are helpful in ensuring appropriate therapeutic response.

Finally, the act of reducing dose due to "toxic level" for well maintained patients or sustaining the present dose for uncontrolled patients because of the threat of overstepping the target range is not proper and certainly would not improve outcome. It should be stressed that outcome for the treatment of epilepsy is complete seizure control with no or minimal side-effects [Brodie & Dichter 1997] and the above treatment methods would eventually defeat the purpose and effectiveness of therapy.

6.5 Limitation of study

The present study design analyses routine data for the determination of therapeutic efficacy on the therapeutic range of phenytoin, carbamazepine, valproic acid and phenobarbitone. This method of data collection is however controversial and did not follow the recommendation by Mattson et al (1982) for prospective evaluation study of efficacy and toxicity of antiepileptic drugs or that for population base studies recommended by Pledger & Schmidt (1994) and can restrict the final conclusions of this study.

Studies using routine patient data that were collected retrospectively have often been stated to be inferior to the traditional study design (prospective, comparative, randomised controlled study etc). The disadvantage of routine data is its reliability and the possibility of being highly biased. The high possibility of bias is mainly due to the effects of unknown concomitant variables that are correlated with included variables. Another problem associated from routine data methodology is data cannot be deliberately constructed as it can in a controlled study design. Questions that are often asked of studies on routine data are those that concern the way data was gathered and whether any control procedures were instituted. Thus, these weaknesses can result in mistakenly concluding that variables do correlate.

It should also be pointed that data that are collected retrospectively do have its advantages. Among the reasons quoted are the absence of restriction for study admittance of patients groups, cheapness, and most importantly it provides a data base of a true patient population that include data on extreme and atypical as well as on well-controlled patients. These atypical cases might be less useful for analysis since it constitute the extraneous factor namely called as 'noise', but the defects in fit serve to suggest further exploration [Sheiner et al 1977].

The weaknesses of routine data collection can also be controlled by a study design that detailed certain restriction for admittance of data [CRD report 4 1996]. For this study, the above limitations were controlled by having inclusion and exclusion criteria for selection of data. Full particulars of patients selected were essential and patients with incomplete or undefined characteristic were omitted. However, the problems of reliability concerning the completeness of recorded information obtained from patients files might be the confounding factor that obstructs the validity of results.

Statistical insufficiency in this study was complemented by the use of odds ratio as a tool to measure effect. Odds ratio is used to observe the difference between controlled and uncontrolled patients within the target range. This ratio is also applicable when pooling data across strata(between ethnic groups) and where the summary effect provide statistical evidence of their relationship. The method offers strength in statistical power by the method of pooling weighted data and the use of Q chi-square statistic to ascertain the relationship between ethnic group and efficiency.

The present study is however not devoid from limitations. One crucial factor that limits its used is it fails to take into account the variation between patient groups. This is clearly evident for adult patient groups on carbamazepine therapy which are unmatched for confounders such as age, weight, or dose. This is another example of a problem that usually beseiged routine data for it is almost impossible to clearly construct a controlled study where matching for either age, weight or dose would definitely lead to a better and a much refined findings. This weaknesses can nevertheless be resolved by the method of stratification where it is essential to ascertain which of the three confounding variables could have contributed to the observed difference.

These difference were however found irrelevant since the pooled odds ratio among the three groups were statistically insignificant. Thus, the results of this study has in fact managed to answer questions raised earlier by defining the 95% confidence interval of each conclusions which is then used in determining its statistical significance.

6.6 Conclusion

This study had managed to verify the current views in the used of therapeutic blood monitoring and the concept of target range of established AEDs for chronic epileptics. The recommendations made by the Commission of AEDs, International League Against Epilepsy (1993) on the guidelines for therapeutic monitoring on established AEDs such as phenytoin, carbamazepine, valproic acid and phenobarbitone was demonstrated to be applicable in the multi-ethnic society of Malaysia.

The above results was confirmed as the differences observed between Malays, Chinese and Indians pooled odds ratio were proven to be statistically insignificant. This finding clearly showed that therapeutic response were no different for all ethnic

groups in the defined therapeutic range. Observed inter-individual variation in all ethnic groups treated with the studied AEDs also proved that treatment should be individualised. This study however could not verify for intra-individual variations.

Age was not found to be an important factor for the association between therapeutic response and the target therapeutic range. This was confirmed by the pooled odds ratio of carbamazepine and valproic acid. Similar observation were not conclusive for phenytoin and phenobarbitone due to inadequacy of data.

It should be mentioned that the results of this study are unique in that no other study had ever reported or compared effectiveness of the current therapeutic range between ethnic groups. This study nevertheless does suffer from certain limitations associated with data commonly gathered from retrospective studies and the use of the odds ratio to measure effect. These weaknesses were however complemented by the introduction of set criteria for data selection and matching groups for confounders to improve accuracy.

Finally, these findings had managed to conclude that routine monitoring of established antiepileptic drugs to that of their target ranges is unimportant and had little bearing to good therapeutic response. Therapeutic response which was measured to the degree of seizure control was not dependent on either age or inter-ethnic differences. These observation is thus in-line with the recommendation outlined by the Commission on Antiepileptic drugs, International League Against Epilepsy (1993).

Chapter 7:

Carbamazepine: Steady-state pharmacokinetics in multi-ethnic epileptic population

7.1 Introduction

Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide) was introduced in the early 1960's and is an effective agent for the control of epileptic seizures. It is now used widely in the treatment of both partial and generalized epilepsy [Troupin et al 1977 and trigeminal neuralgia [Tomson et al 1980].

Data on pharmacokinetic relationship between dose and serum level are currently inconclusive either in monotherapy or polytherapy. In chronically treated patients, a poor relationship between dose and serum level has often been reported [Nolen et al 1988, Bertilsson 1978, Morselli 1977, Pynnonen et al 1977, Johannessen and Strandjord 1975, Mihaly et al 1977, Christiansen and Dam 1977] while Perruca et al observed a linear relationship. Others reported a curvilinear relationship [Kudriakova et al 1992, Eadie and Tyrer 1980, Rambeck et al 1987] and this finding was associated with the induction by carbamazepine of its own metabolism(autoinduction) [Cloyd et al 1980, Levy and Kerr 1988].

The controversy on the non-linear relationship between clearances and dose has been supported by several authors [Huf & Schain 1980, Rane et al 1978, Schain et al 1977]. Nevertheless, Summers and Summers (1989) and Kudriakova et al (1992) found apparent clearances of carbamazepine to be linearly related to dose. Summers and Summers (1989) further mentioned the probability that the genetic makeup could affect the clearance and dose relationship.

Inter-ethnic differences in carbamazepine metabolising capacity have yet to be accounted for in its pharmacokinetic interindividual variations. Recently, studies by Lin et al (1991) and Yoon et al (1996) reported that the half-lives of carbamazepine in Chinese and Koreans were in fact significantly different from Caucasians. Lin et al (1991) further concluded that the time course for autoinduction for Chinese is about 46 days and this duration is higher than the 21 to 30 days reported by Bertilsson et al (1986) and Mikati et al (1989) for European based patients.

Objectively, this study has three main goals. Firstly, it aims to investigate the relationship between carbamazepine daily dose and serum level. The second objective is to determine the correlation between apparent clearance and dose (dose-dependent relationship). Finally, to examine the influence of ethnicity on the two earlier relationship in the multiethnic Malaysian society.

7.2 Methods

i. Patients

The data used for this study were obtained retrospectively from outpatients and inpatients treated for epilepsy in five hospitals on the Western Peninsula of Malaysia. A total of 549 randomly selected dose and serum concentration pairs from 410 adult and 139 paediatric epileptic patients were included in the study. The method of random selection of dose and serum pairs is decribed in appendix 5-10.

Table 7-1: Summary data of adult patients

Characteristic	Malays	Chinese	Indians
No. of patients	130	165	115
Ratio of male/female	55:77	92:83	56:59
Age(yr)			
Mean,sd	33.21,9.69	36.85,12.25	33.09,10.47
Range	18.00-63.00	18.00-72.00	18.00-62.00
Weight			
Mean,sd	58.23,10.49	58.98,10.82	54.59,10.84
Range	34.00-90.00	36.00-87.00	31.00-78.00
Disease duration(yr)			
Mean,sd	13.38,10.49	14.54,12.14	14.23,10.64
Range	0.75-44.00	1.00-61.00	0.75-40.00
Epilepsy Types			
Generalised	93	126	106
Partial	23	38	8
Others	14	11	1
Dose(mg/kg/day)			
Mean, sd	10.94,6.30	12.76,5.92	13.21,6.45
Range	1.25-32.50	2.53-32.00	3.23-34.88
Css(mg/L)			
Mean,sd	6.42,2.18	6.63,2.03	6.68,1.91
Range	1.42-12.24	2.60-13.65	3.00-11.25
CL(ml/min/kg)			
Mean,sd	1.17,0.54	1.32,0.46	1.42,0.60
Range	0.23-2.76	0.28-3.10	0.46-3.06

These patients were divided into groups according to age and ethnicity. The three ethnic groups studied were Malay, Chinese and Indian and these patients were subdivided further according to age. Patients were categorised as paediatric or adult based on the age limit of 18 years old. The clinical characteristics of the patients pertinent to the study are presented in table 7-1 and 7-2.

Table 7-2: Summary data of paediatric patients

Characteristic	Malays	Chinese	All patients
No. of patients	62	43	34
Ratio of male/female	38:24	24:19	13:21
Age(yr)			
Mean,sd	10.61,4.57	11.52,4.21	12.32,3.37
Range	1.00-17.00	4-17.00	5.00-17.00
Weight			
Mean,sd	30.67,12.13	37.15,14.77	35.71,11.65
Range	5.10-58.00	16.00-80.00	10.30-57.00
Disease duration(yr)			
Mean,sd	6.57,4.50	5.13,3.65	6.21,3.15
Range	0.50-16.00	0.50-14.00	2.00-12.00
Epilepsy Types			
Generalised	41	27	21
Partial	9	10	11
Others	12	6	2
Dose(mg/kg/day)			
Mean, sd	15.69.12.73	14.80,8.50	16.28,6.57
Range	2.86-75.00	2.50-40.00	7.02-33.33
Css(mg/L)			
Mean,sd	5.84,2.16	5.53,2.12	6.70,1.86
Range	1.98-13.02	1.71-10.05	2.35-10.70
CL(ml/min/kg)			
Mean,sd	1.89,1.40	1.85,0.91	1.75,0.72
Range	0.65-8.39	0.46-5.27	0.74-3.94

The patients received carbamazepine alone or in combination with other antiepileptics. Non-compliant patients were determined from patient notes and plasma concentration request forms and were excluded. Details pertaining to each dose and serum concentration selected were checked from patient notes for confirmation. Only serum concentration after at least 30 days of therapy were taken by which time steady state could be assumed and autoinduction was stabilised [Bertillson et al 1980, Browne et al 1987, McNamara et al 1978]. The serum level was measured by the fluorescence polarization immunoassay(FPIA) method. The coefficient of variation of this assay was less than 10%. The retrospective study was approved by the Head Director of Health, Ministry of Health with the cooperation of Chief Director of Pharmaceutical Services and Director of all

hospitals involved. Further details of the retrospective study are described in chapter 5 of this thesis.

ii. Analysis

Relationship between dose and serum level was determined by linear regression analysis. Regression lines were calculated by least square regression and correlation coefficients were calculated. To ease calculation, MINITAB version 10 were used for all calculation. A P value less than or equal to 0.05 was considered significant. The parameter used for analysis was dose, serum level, and apparent clearance. Doses were expressed in either milligram per day(mg/day) or per kilogram(mg/kg), serum levels in milligram per liter(mg/L) and clearance in Liters per kg per day(L/kg.day).

The formula by Wagner (1965) was used to calculate apparent clearance and is described in chapter 1, section 1.5.5, i.e,

$$Css_{ij} = \frac{F.D_{ij}}{V_{ij}.K_{ij}.\tau} \qquad \textit{(equation 7.1)}$$

where Css_{ij} is the steady state blood concentration, D_{ij} is the dose, τ is the length of the dosage interval, K_{ij} is the first-order rate constant for overall loss of drug from the blood, F is the fraction of each dose which is absorbed(bioavailability factor), and V is the apparent volume of distribution. Plotting Css and D_{ij} would be a straight line with the y intersect of 0 and the assumption that the other variables remains constant.

Since clearance (*CL, Liters/day*) is equal to *Vij multiplied by Kij* , the formula can be rewritten as;

$$\textit{Clearance(CLij)} = \frac{F.Dij}{Css_{ij}.\tau} \qquad \textit{(equation 7.2)}$$

τ can be in minutes or hours per day. Ismail & Rahman (1993) had earlier reported the F value for Malaysian epileptic to be 0.96. For simplicity in calculation, F is however taken as equal to 1.

Carbamazepine clearance was calculated on the assumption that at steady state (at equilibrium) the ratio of change in dose is proportionate to the ratio of change of serum concentrations. Clearance is measured in liters per kg.hour (L/kg.hr). Correlation of clearance and dose were evaluated by linear regression.

To ascertain inter-ethnic differences, the method proposed by Greenland (1971) in estimating trend from the slope of regression equation was employed. Detailed outline of the formulas used was described in chapter 3, section 3.3.3(b). Differences in age, sex, disease duration and epilepsy types in each group were evaluated by one way analysis of variance(ANOVA) while the coefficient of variation(CV) between serum level/dose ratios was used to differentiate for inter-individual variation. The formula to calculate coefficient of variation(CV,%) = (SD ÷ Mean) X 100, where SD denotes standard deviation.

7.3 Results

Analysis of variance of variables of adult patients found that age($p<0.01$), weight($p<0.01$) and dose($p<0.01$) differed significantly between ethnic groups. Mean length of disease years and serum concentration were similar. Mean dose($p<0.05$), serum level($p<0.05$) and weight($p<0.05$) were significantly different in paediatric patients. Length of disease years and age did not show any statistical differences (Table 7-3).

7.3.1 Relationship between dose and serum level

Table 7-4 shows the correlation between dose in mg/day and mg/kg with serum levels in adult and paediatric patients. Overall, there was evidence of a rather weak linear relationship between dose in mg/day and serum level being better correlated than dose expressed in mg/kg for all ethnic groups. These differences was more visible in the paediatric than in adults patients where all three ethnic group showed a higher correlation coefficient for dose prescribed in milligram per day. The possible reason for this observation could be due to all of the paediatric patients were on long term carbamazepine therapy where dosing is based on therapeutic response rather than weight.

Table 7-3: Analysis of variances of variables

Variables	Malay (mean,sd)	Chinese (mean,sd)	Indian (mean,sd)	p-value (mean,sd)
1. Adult				
-Age(years)	33.21,9.69	36.85,12.25	33.09,10.48	0.003
-Weight(kg)	58.23,11.63	58.98,10.82	54.59,10.84	0.003
-Length of disease years(years)	13.38,10.49	14.54,12.14	14.23,10.65	0.666
-Dose(mg/day)	605.40,295.00	718.30,293.40	690.40,304.60	0.004
-Serum concentration(mg/L)	6.42,2.18	6.63,2.03	6.68,1.91	0.553
2. Children				
-Age(years)	2.81,0.72	2.95,0.82	2.94,0.69	0.538
-Weight(kg)	30.67,12.13	37.15,14.77	35.71,11.65	0.029
-Length of disease years(years)	6.57,4.50	5.13,3.65	6.21,3.16	0.179
-Dose(mg/day)	421.80,263.90	499.50,248.40	552.90,218.80	0.040
-Serum concentration(mg/L)	5.84,2.16	5.52,2.12	6.70,1.86	0.044

Table 7-4: Dose and serum level(SL) relationship in adult paediatric patients

Predictors	Malays	Chinese	Indians
I. Adults			
a) Regression equation (dose,mg/kg)	SL=4.29 + 0.194Dose	SL=3.74 + 0.226Dose	SL=4.66 + 0.153Dose
R-square(adj)	31.6%	43.6%	26.6%
F value	59.23	133.59	40.99
p-value	0.00	0.00	0.00
b) Regression equation (dose,mg/day)	SL=3.71 + 0.0045Dose	SL=3.35 + 0.0046Dose	SL=4.50 + 0.0031Dose
R-square(adj)	36.8%	43.7%	25.3%
F-value	74.40	134.32	38.35
p-value	0.00	0.00	0.00
II. Children			
a) Regression equation (dose,mg/kg)	SL=4.94 + 0.057	SL=3.44 +0.141Dose	SL=4.81 +0.116Dose
R-square	11.5%	31.8%	16.9%
F-square	7.81	19.15	6.51
p-value	0.01	0.00	0.02
b) Regression equation (dose,mg/day)	SL=4.14 +0.0040Dose	SL=2.65 + 0.0057Dose	SL=3.91 + 0.0050Dose
R-square	24.3%	45.6%	35.5%
F-value	19.24	34.34	17.56
p-value	0.00	0.00	0.00

Dose and serum level relationship also displayed a wide scatter in data points along the regression line(figure 7-1 to 7-6). This relationship can implies that there is high inter-individual variation and can be clearly demonstrated by the observed coefficient of variation of serum level/dose ratios for paediatric and adult Malay, Chinese and Indian of 54.45%, 45.41%, 47.19% and 50.06%, 56.48%, 37.83%

Figure 7-1: Carbamazepine dose and serum level relationship in Malay paediatric patients

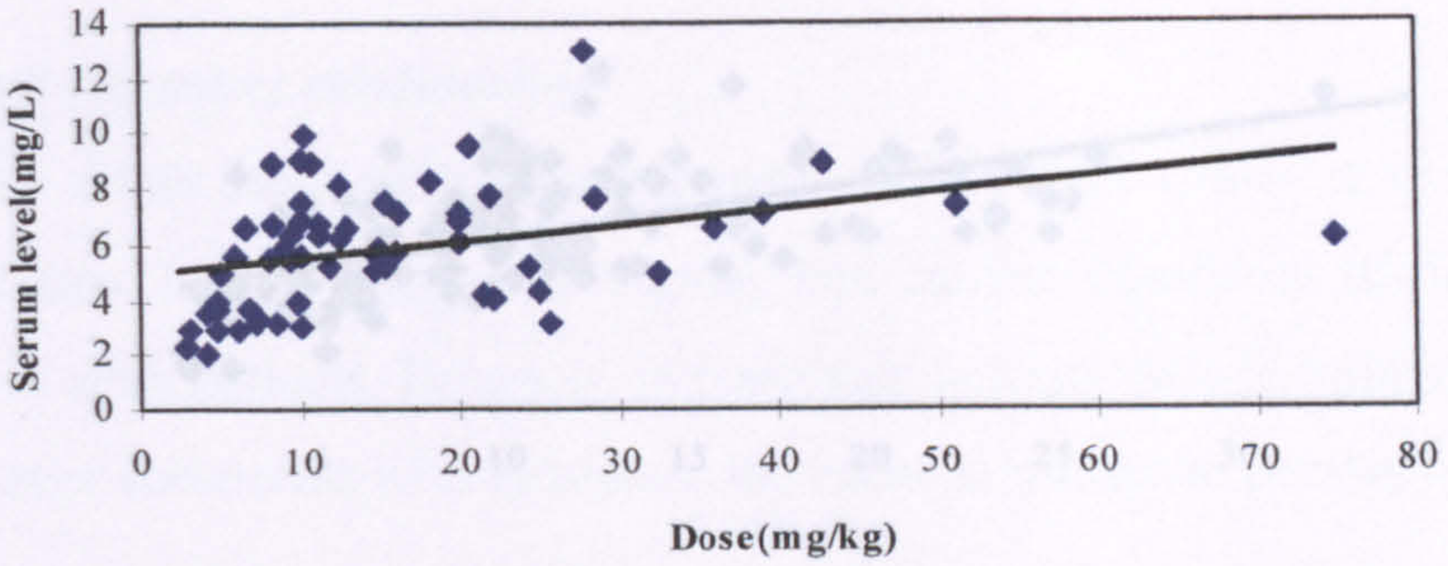


Figure 7-2: Carbamazepine dose and serum level relationship in Chinese paediatric patients

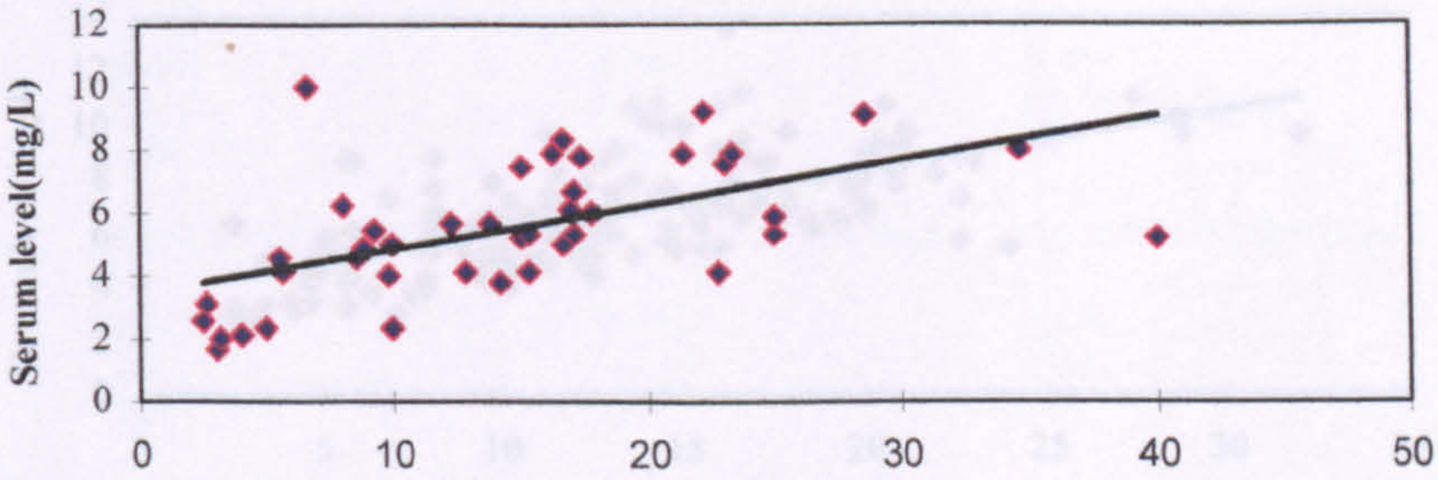


Figure 3: Carbamazepine dose and serum level relationship in Indian paediatric patients

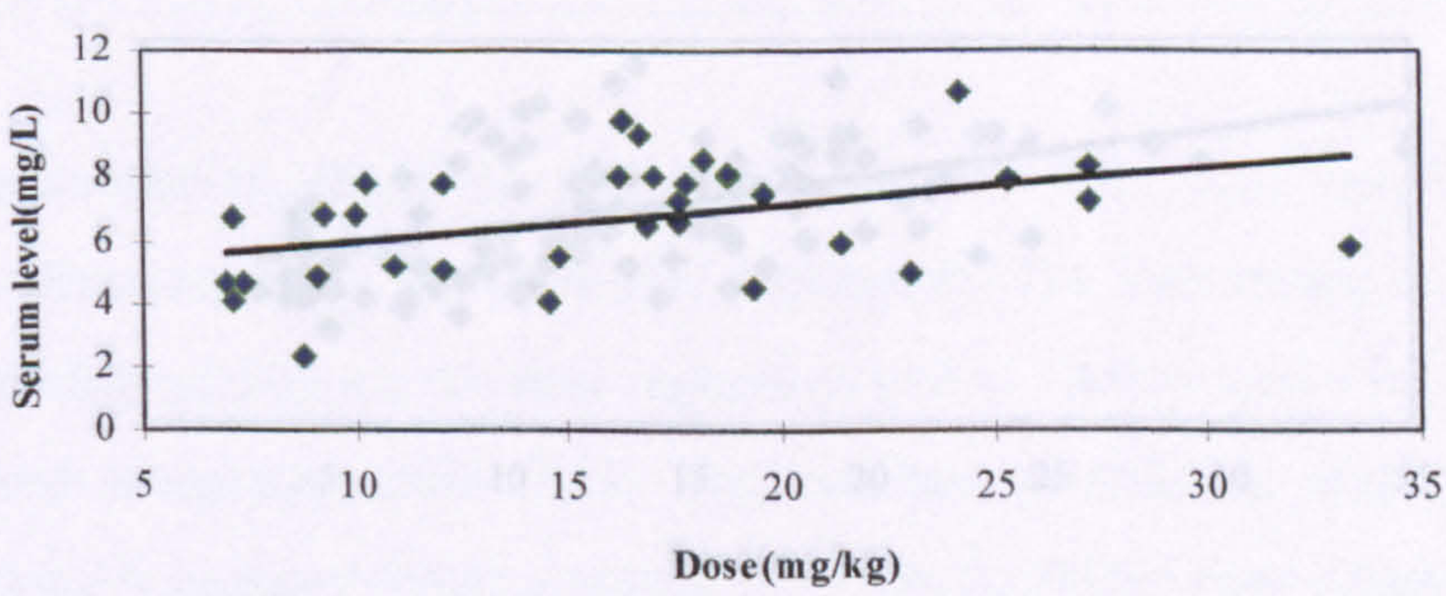


Figure 7-4: Carbamazepine dose and serum level relationship in adult Malay patients

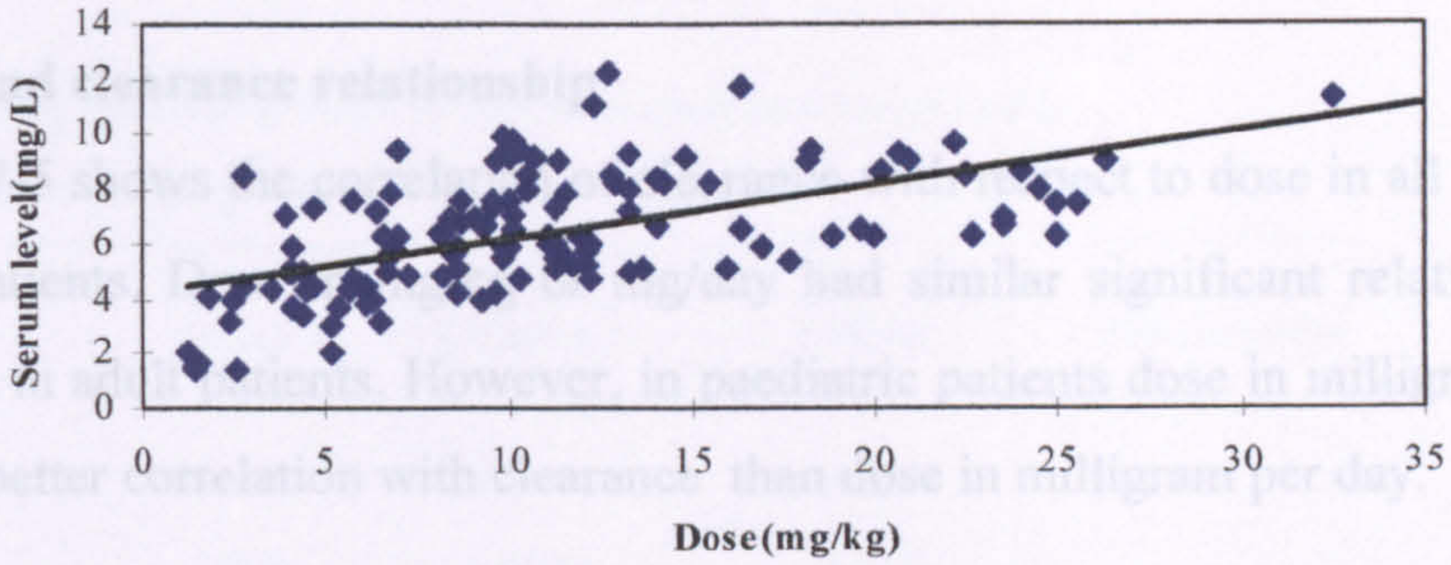


Figure 7-5: Carbamazepine dose and serum level relationship in adult Chinese patients

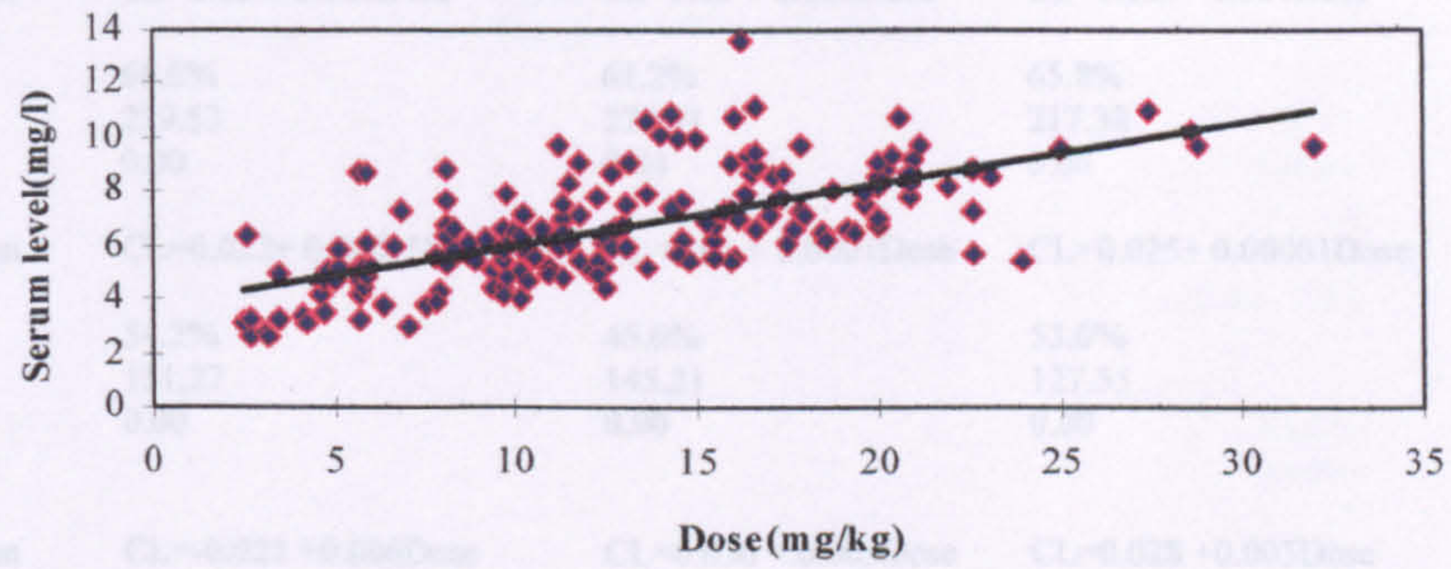
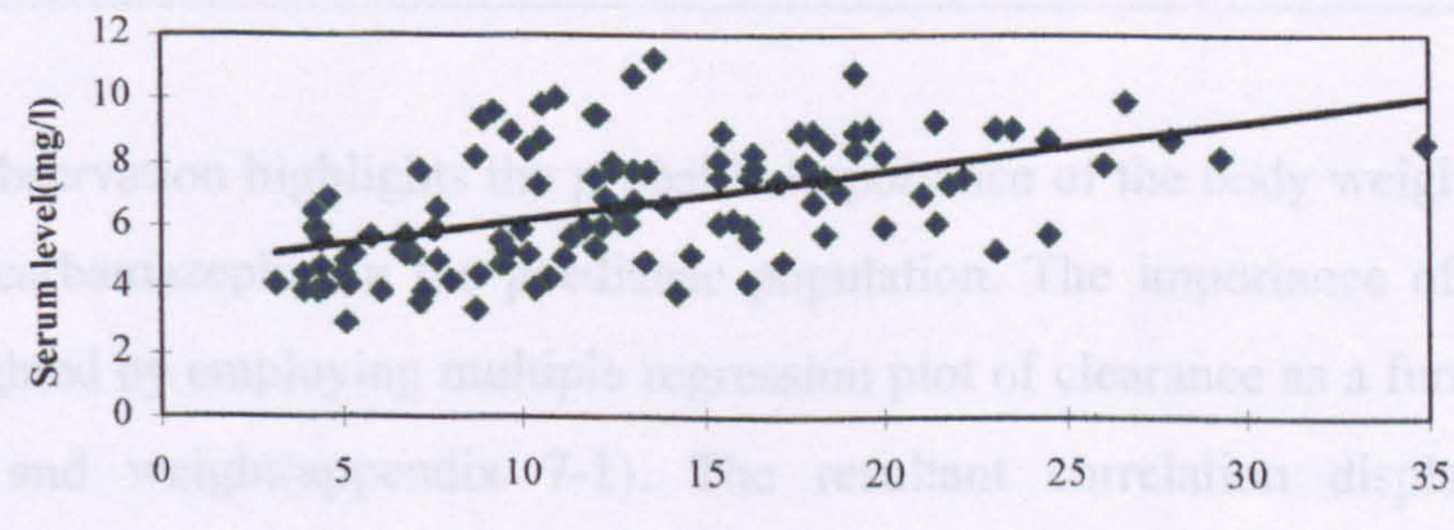


Figure 7-6 : Carbamazepine dose and serum level relationship in adult Indian patients



respectively. These results also suggest that prediction of serum levels based solely on either daily dose or dose per kilogram is not possible.

7.3.3 Dose and clearance relationship

Table 7-5 shows the correlation of clearance with respect to dose in all groups of epileptic patients. Dose in mg/kg or mg/day had similar significant relationship with clearance in adult patients. However, in paediatric patients dose in milligram per kilogram had better correlation with clearance than dose in milligram per day.

Table 7-5: Dose and clearance(CL) relationship in adult paediatric patients

Predictors	Malays	Chinese	Indians
I. Adults			
a) Regression equation (dose,mg/kg)	CL=0.02 + 0.004Dose	CL=0.03 + 0.004Dose	CL=0.025+ 0.004Dose
R-square(adj)	68.6%	61.2%	65.8%
F value	279.53	272.91	217.38
p-value	0.00	0.00	0.00
b) Regression equation (dose,mg/day)	CL=0.022+ 0.0001Dose	CL=0.03 + 0.0001Dose	CL=0.025+ 0.00001Dose
R-square(adj)	54.2%	45.6%	53.0%
F-value	151.27	145.21	127.55
p-value	0.00	0.00	0.00
II. Children			
a) Regression equation (dose,mg/kg)	CL=-0.021 +0.006Dose	CL=0.036 +.0.005Dose	CL=0.028 +0.005Dose
R-square(adj)	78.6%	61.6%	49.7%
F-square	225.43	68.36	33.64
p-value	0.00	0.00	0.00
b) Regression equation (dose,mg/day)	CL=0.038+ 0.0002Dose	CL=0.075+ 0.0001Dose	CL=0.077+ 0.00005Dose
R-square(adj)	31.5%	9.1%	3.3%
F-value	27.60	5.22	2.12
p-value	0.00	0.03	0.15

This observation highlights the probable importance of the body weight-factor in dosing of carbamazepine in the paediatric population. The importance of weight can be highlighted by employing multiple regression plot of clearance as a function of dose(mg/kg) and weight(appendix 7-1). The resultant correlation displayed an increase of about 2% in correlation(r_{adj} square = 73.3%, p =0.00) than clearance and dose(r_{adj} square = 71.6%, p = 0.00) alone. However, the smaller value of S (standard

deviation to the regression line) and F (F-ratio) of the latter to that of the former could be due to disparities in study design rather than a real relationship.

Table 7-6: Summary estimate of slope of dose and clearance relationship in various ethnic groups

Age-group	Malay	Chinese	Indian	Final results
I. Adult				
-slope	0.00435	0.00367	0.00423	
-variance	0.0041	0.00370	0.0049	
-weights	243.90	270.27	204.08	
-Summary slope	-	-	-	0.0041
-Q statistic				0.0001
-df				2
-p				0.50
-95% C.I				-0.07 - 0.08
II.Paediatric				
-slope	0.00588	0.00506	0.00473	
-variance	0.0081	0.0017	0.0084	
-weights	123.46	588.23	119.05	
-Summary slope	-	-	-	0.0051
-Q-statistic				0.0001
-df				2
-p				0.50
-95% C.I				-0.06 - 0.07
iii. All				
-Summary slope	-	-	-	0.0046
-Q statistic				0.0006
-df				5
-p				<0.000
-95% C.I				-0.045 - 0.054

The highly correlated relationship between clearance and dose per kilogram distinctly showed that clearance increases proportionally with increase dose. This observation confirmed that carbamazepine exhibited dose-dependent properties(figure 7-7 to figure 7-12).

7.3.3 Influence of age

Pooled summary estimate of slope obtained from the relationship between clearance and dose among paediatric and adult patients (table 7-5) showed that there existed a definite and significant difference($p > 0.001$). These observations demonstrate that age had a significant influence on carbamazepine clearance. Pooled

Figure 7-7: Carbamazepine dose and clearance relationship in Malay paediatric patients

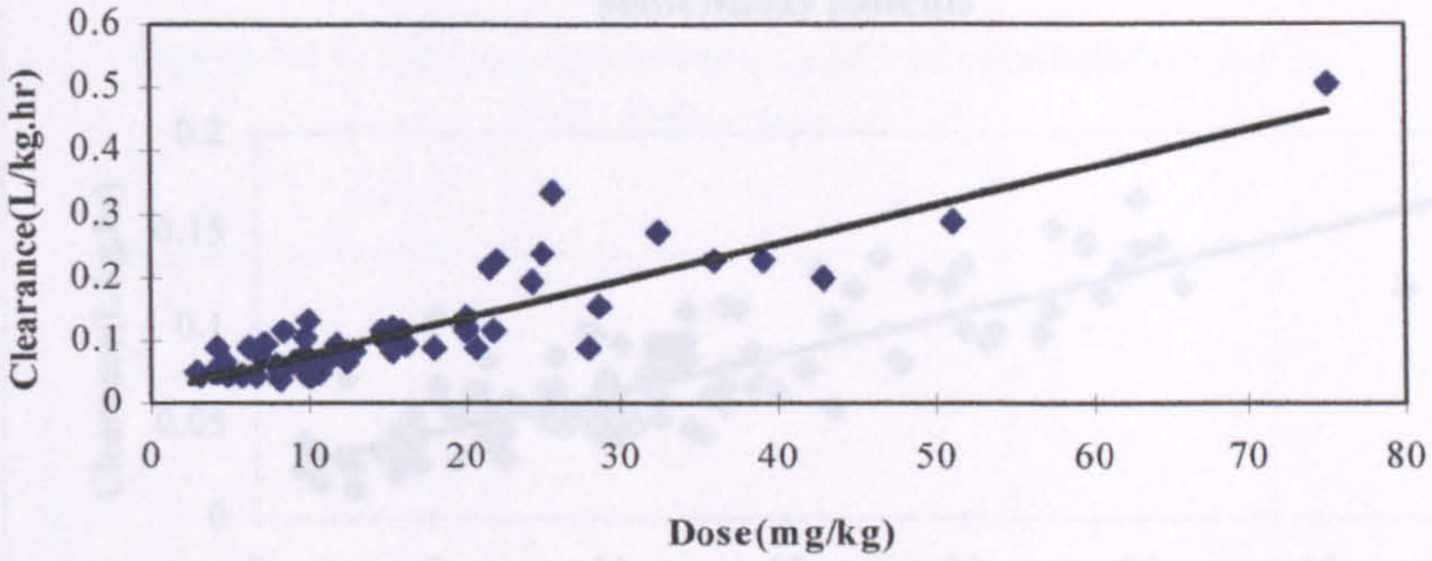


Figure 7-8: Carbamazepine dose and clearance relationship in Chinese paediatric patients

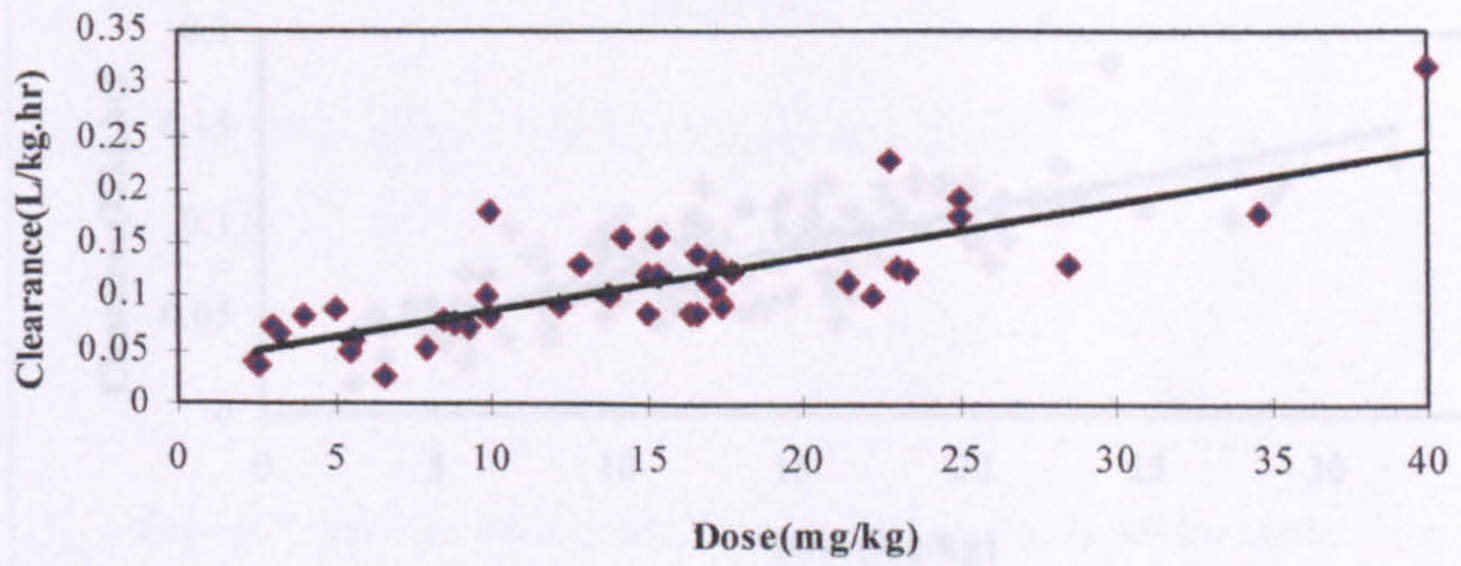


Figure 7-9 : Carbamazepine dose and clearance relationship in Indian patients

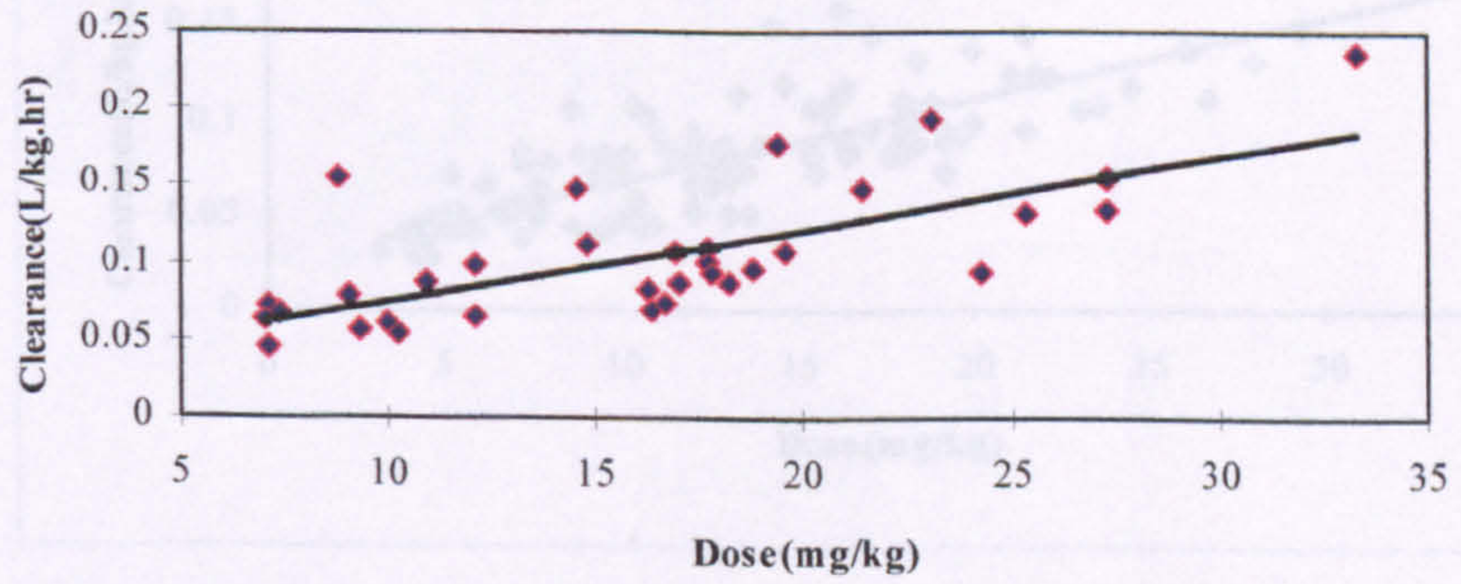


Figure 7-10: Carbamazepine dose and clearance relationship in adult Malay patients

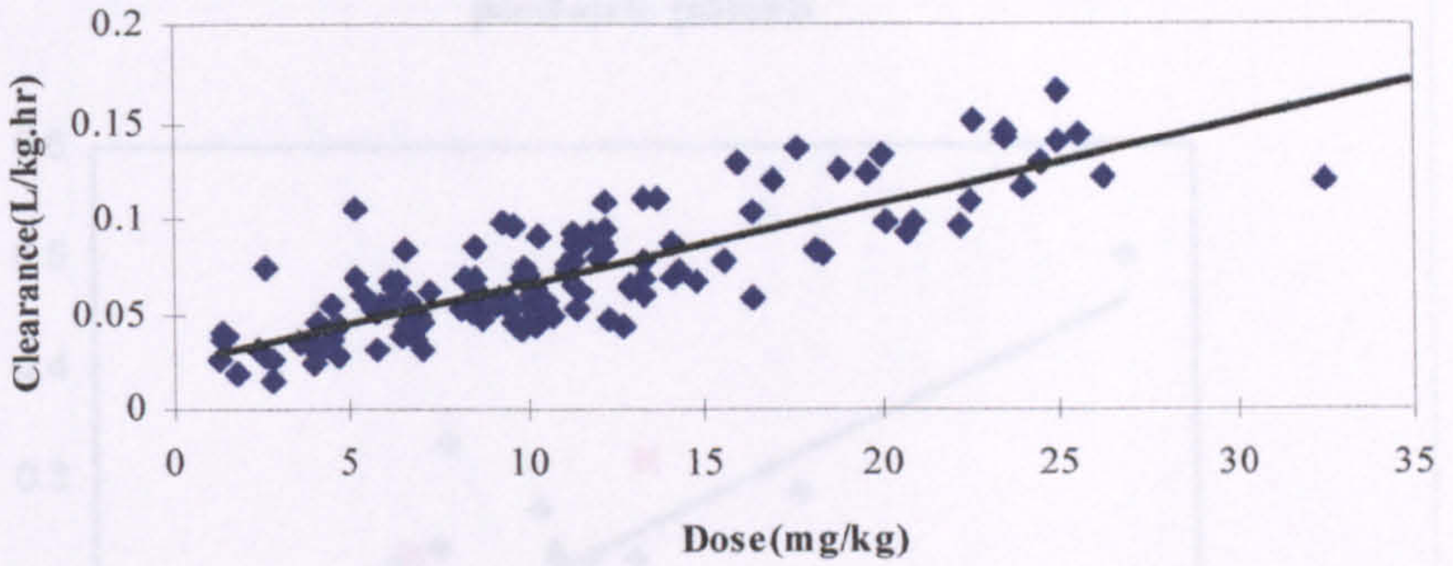


Figure 7-11: Carbamazepine dose and clearance relationship in adult Chinese patients

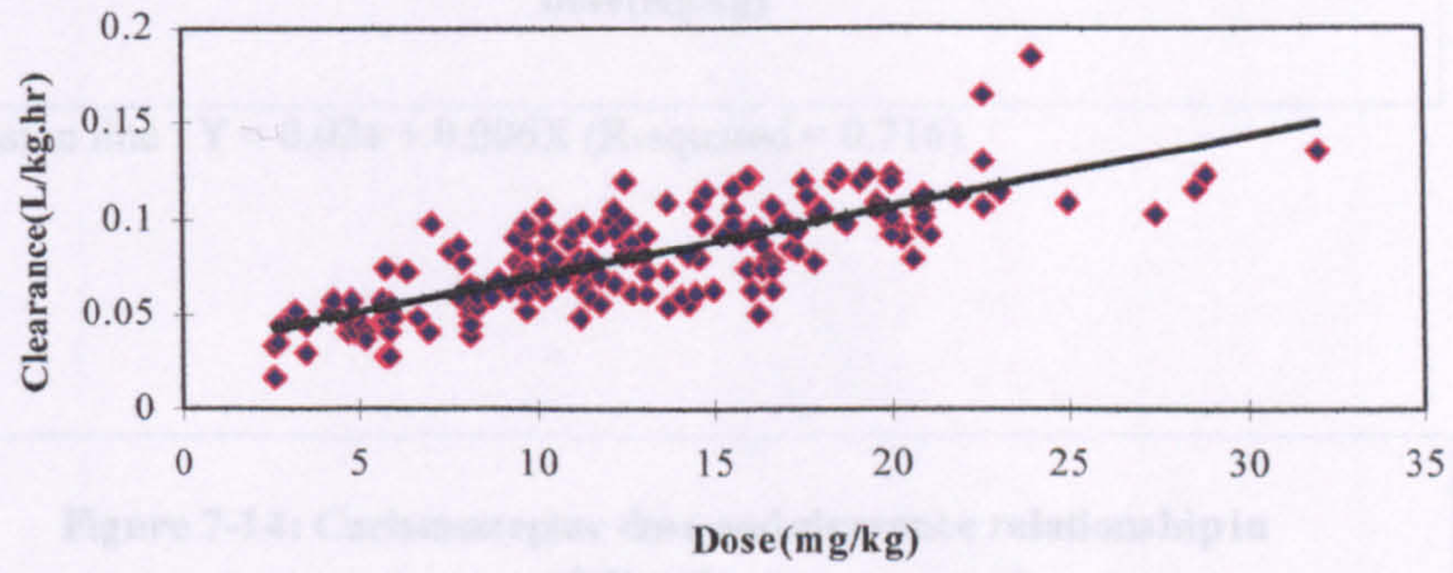


Figure 7-12: Carbamazepine dose and clearance relationship in adult Indian patients

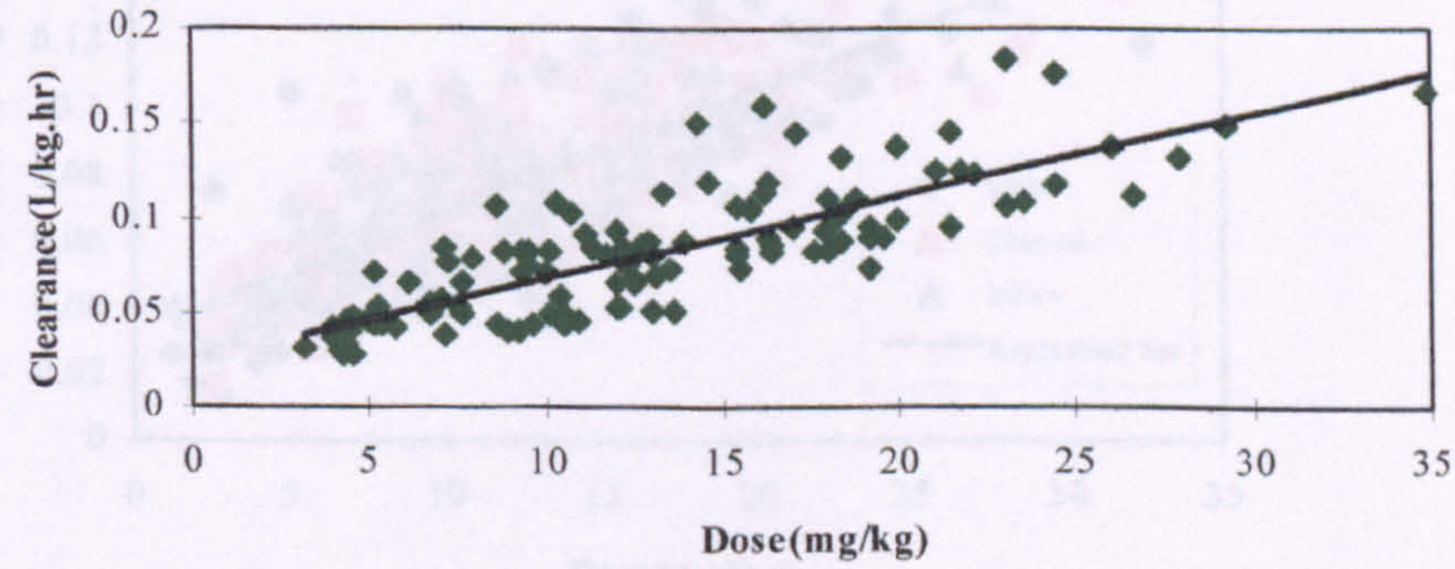
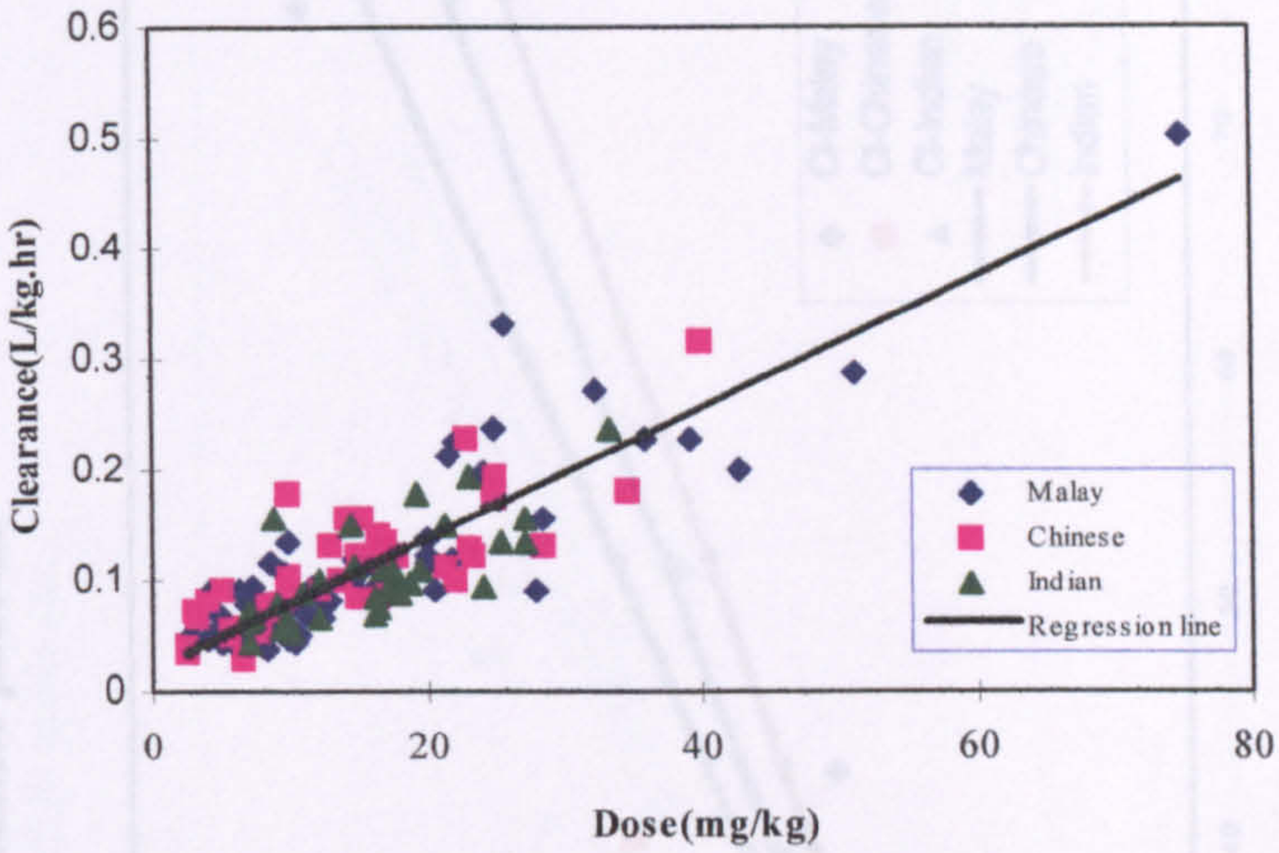
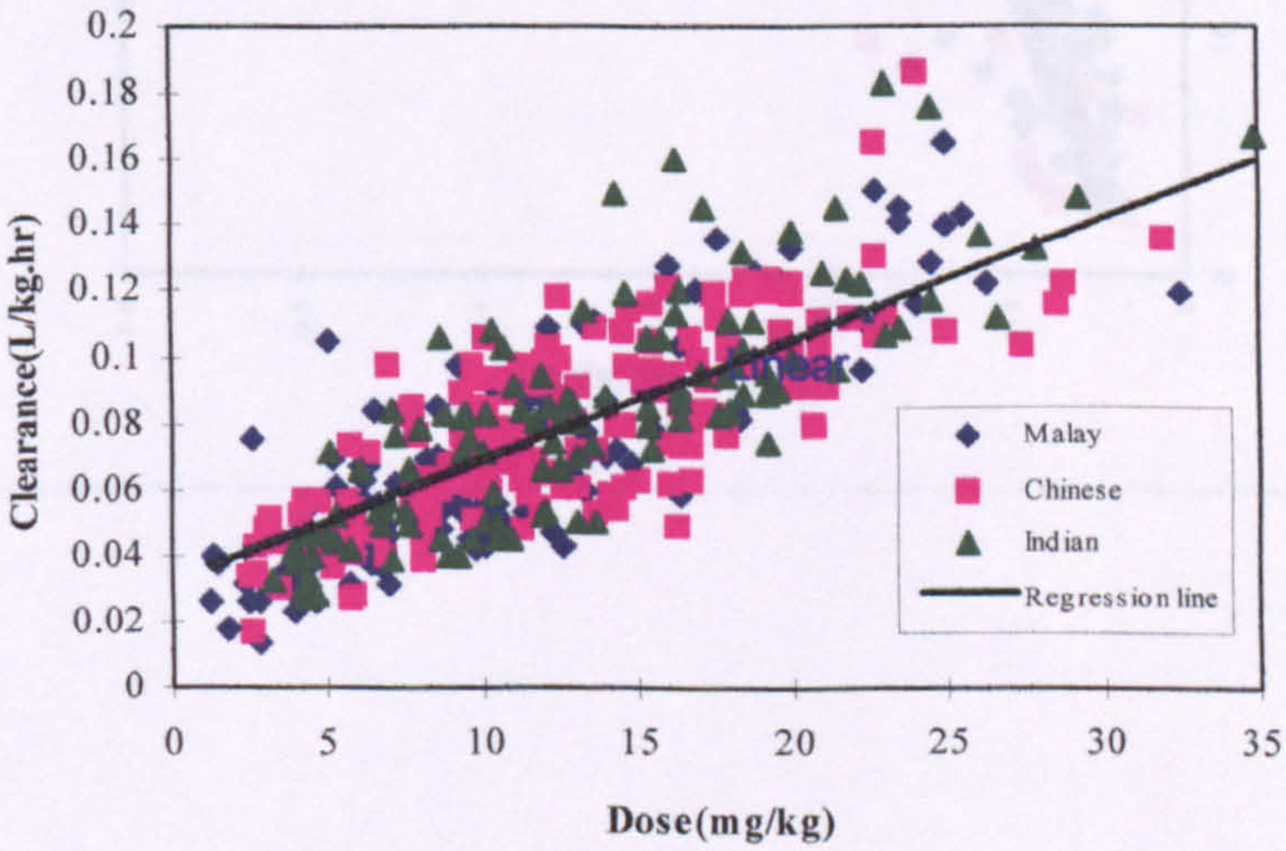


Figure 7-13: Carbamazepine dose and clearance relationship in paediatric patients



Regression line : Y = 0.024 + 0.006X (R-squared = 0.716)

Figure 7-14: Carbamazepine dose and clearance relationship in adult patients



Regression line : Y = 0.03 + 0.004X (R-squared = 0.656)

Figure 7-15: Ethnic differences of carbamazepine dose and clearance relationship in paediatric patients

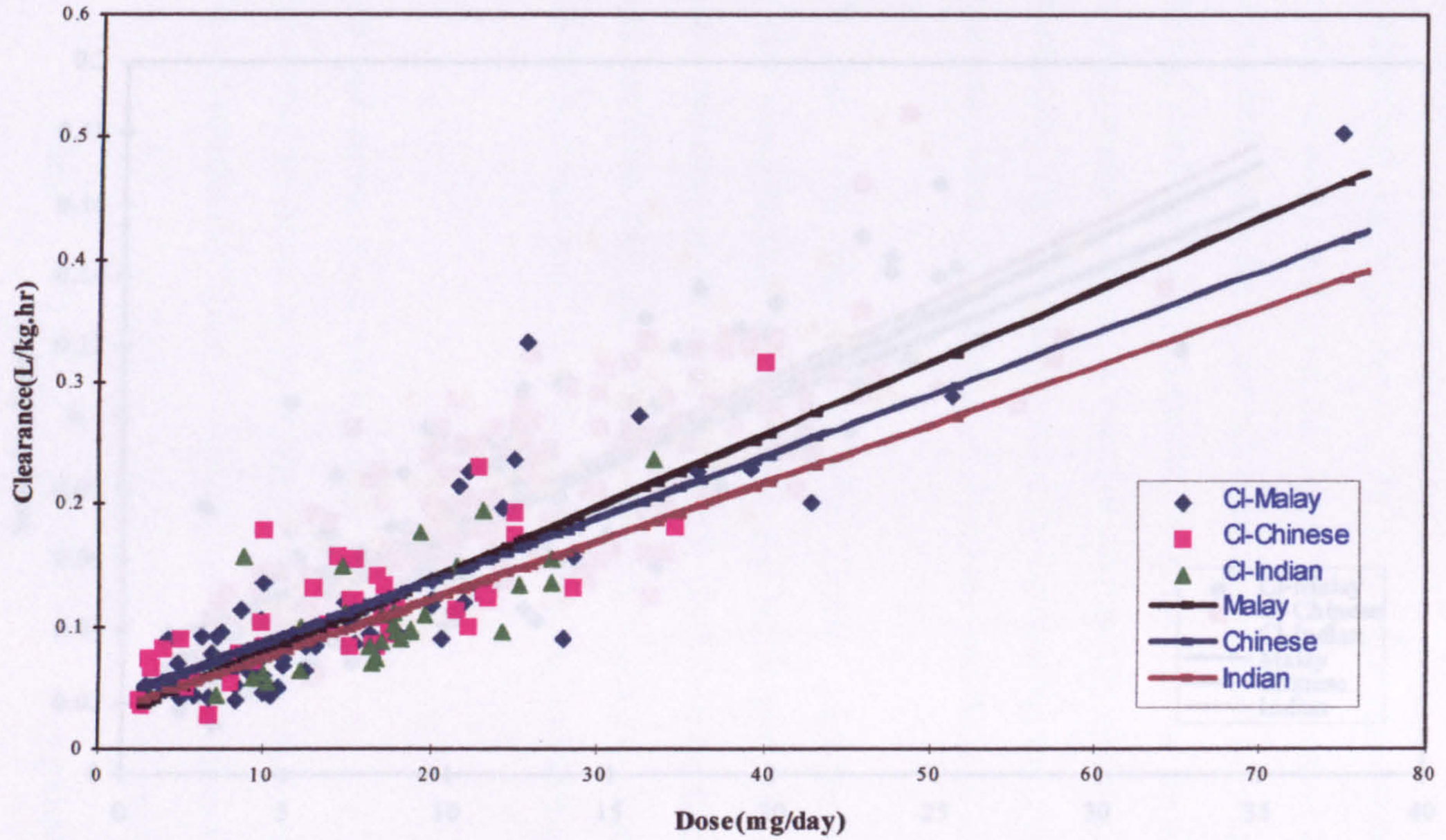
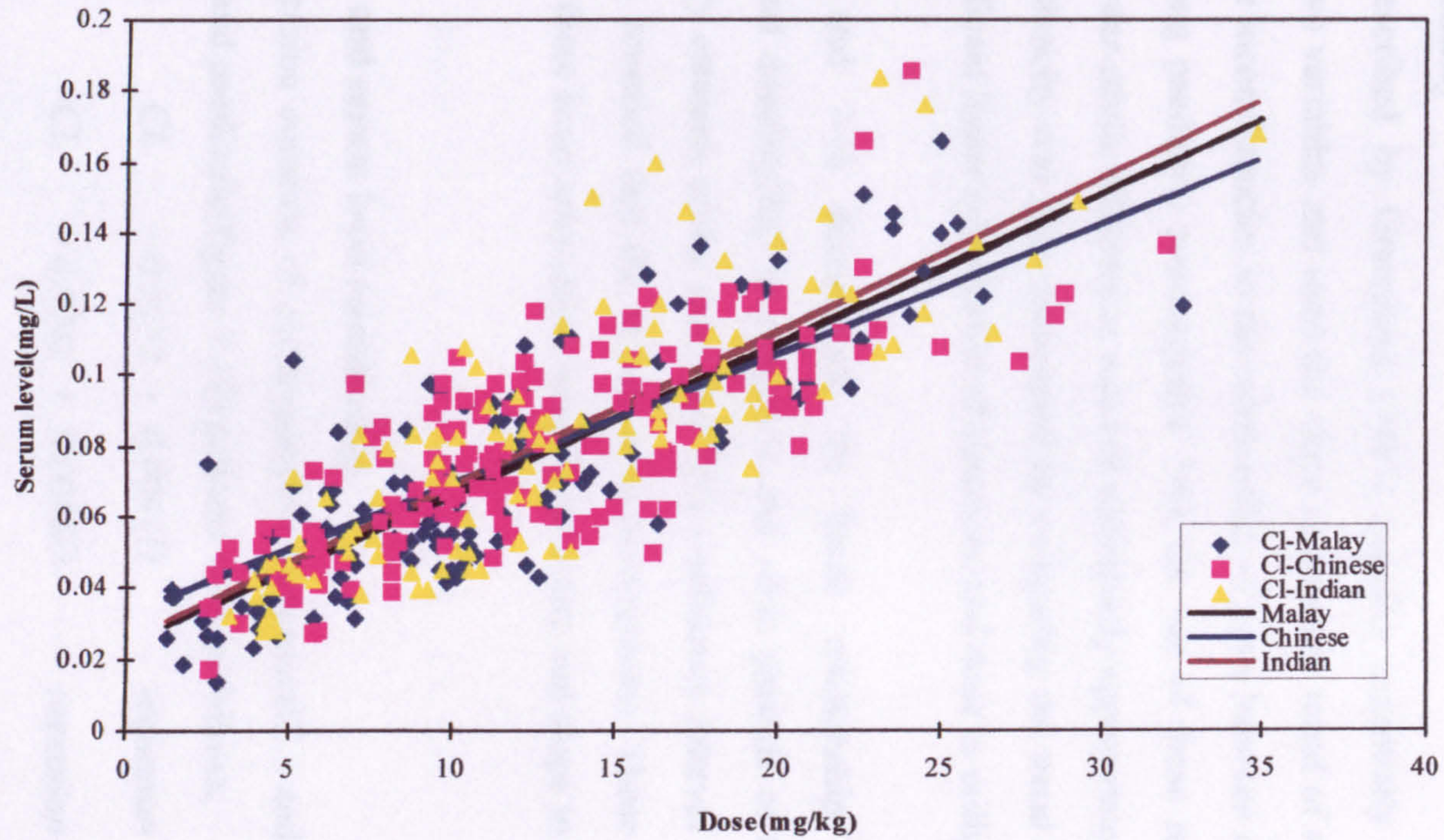


Figure 7-16: Ethnic differences of carbamazepine dose and clearance relationship in adult patients



estimate of slope of children and adults showed that the clearance of paediatric patients was significantly higher than adult patients(figure 7-13, figure 7-14).

7.3.4 Influence of ethnicity

The method described by Greenland (1987) requires essentially a linear relationship between two variables and used the slope to estimate trend of measured effect. Since there were inconsistencies in the relationship of plots between clearance and dose(mg/day) among paediatric patients(table 7-6), the use of these regression equations to establish inter-ethnic differences was not statistically appropriate and was unjustified. Effect of ethnicity was thus determined by comparing the trend from the more reliable and significant linear relationship of clearance and dose in milligram per kilogram body weight.

Figures 7-15 and 7-16 demonstrate the linear relationship between clearance(L/kg.hour) and dose(mg/kg) in paediatric and adult patients of the three ethnic groups. Summary estimate of the slope and 95% confidence interval in adults and paediatric patients revealed that the slopes were homogenous. These findings distinctly signifies that there is no association between ethnicity and slope in both age groups.

7.3.5 Predicted dose and serum level relationship

The linear regression equation of carbamazepine clearance(CL) and dose(D) for adults(figure 7-13) and paediatric(figure 7-14) patients were as follows;

Adults ; $CL = 0.0272 + 0.0041D$ *(equation 7-3)*

Paediatrics ; $CL = 0.0241 + 0.0056D$ *(equation 7-4)*

The units used for clearance was liters per kilogram per hour and dose in milligram per kilogram body weight.

Relationship of dose(D) and serum levels at steady-state(Css) can be derived by combining equation 7-1 and equations 7-3 or 7-4. The latter two equations had to be transform to litres per kg per day by a multiple of 24. The final equations for paediatric and adult were;

Figure 7-17: Predicted carbamazepine dose and serum level relationship in adult patients

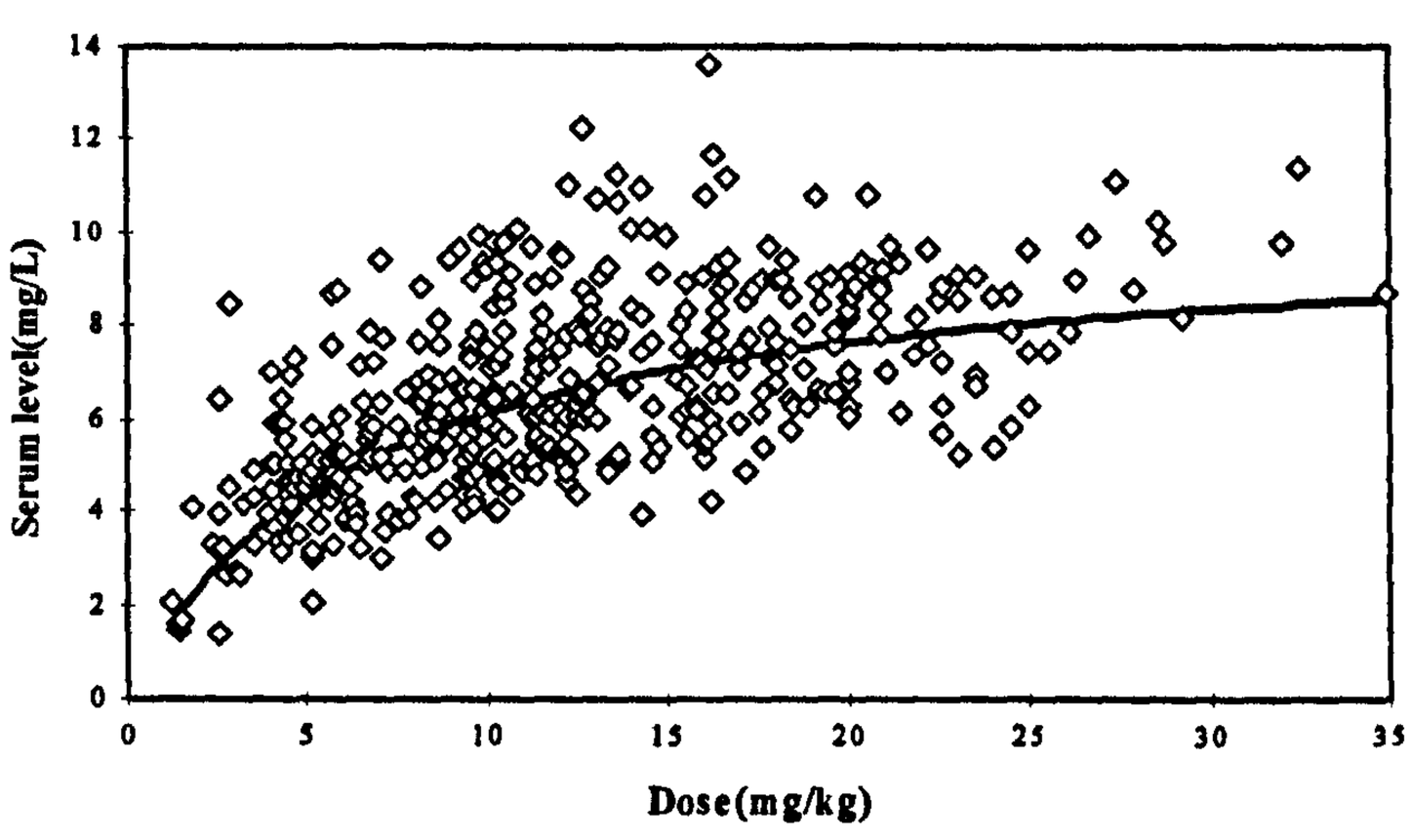
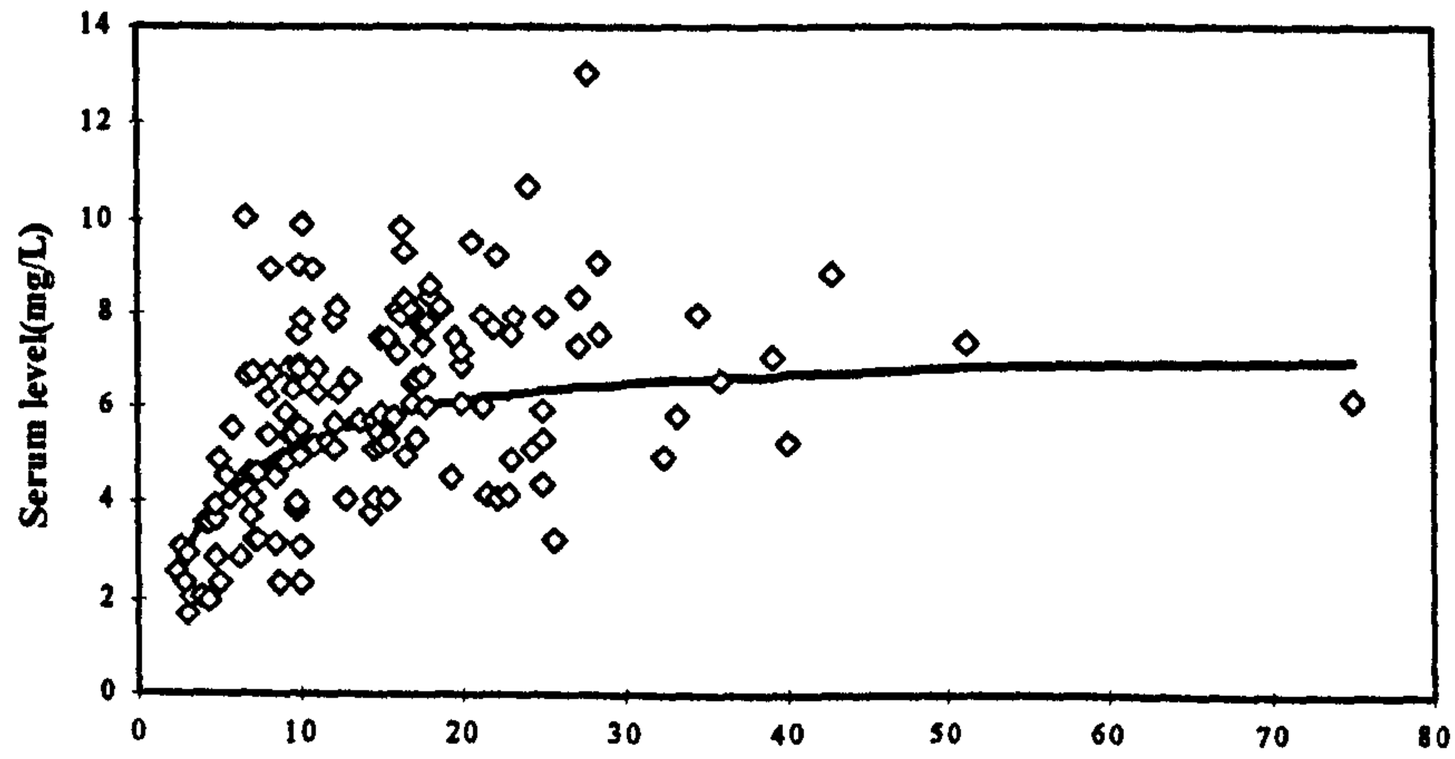


Figure 7-18: Predicted carbamazepine dose and serum level in paediatric patients



$$\textit{Adults} \quad ; \quad Css = \frac{10.16D}{6.53 + D} \qquad \textit{(equation 6-4)}$$

$$\textit{Paediatric} \quad ; \quad Css = \frac{7.69D}{4.46 + D} \qquad \textit{(equation 6-5)}$$

These equations theoretically resemble a hyperbolic association and thus a non-linear relationship. The curves reflecting these equations are presented in figures 7-17 and 7-18.

7.4 Discussion

The design of this retrospective study is not at all the best suited to determine population-based relationship between dose and serum levels of any drug. Although a prospectively designed study with frequent drug level analysis would be more desirable, problems of cost, time, study size and unspecific selection of patients groups are the hindering factors for any population studies [Sheiner et al 1977]. Reports of carbamazepine population based studies employing both retrospective [Summers & Summers 1989] and prospective [Kudriakova et al 1992, Bernus et al 1996] patients data are few and only the recent introduction of sophisticated statistical software such as NONMEM [Sheiner & Beal 1981] has led to more studies being reported [Odani et al 1996, Yukawa 1995, Delgado Iribarnegaray et al 1997].

These earlier population base studies pooled data from patients with unequal number of dose and serum level pairs in investigating the relationship. This method is commonly known as the naive pooled approach and did not allow for inter-individual variability [Sheiner & Beal 1980], and hence the degree of accuracy of the final results is doubtful. It should be mentioned that during regression, patients with more dose and serum level pairs have higher influence on the overall conclusion.

However, the present study used a random selection of a single dose-serum level pair from each patient, being more appropriate in determining relationship. By this method, similar numbers of data points from each patient provide an equal contribution during regression analysis and the final results are freer of any possibility of being biased. This method would thus enhance the statistical validity of the overall relationship. Similar studies using single set of dose/serum level pairs of either the

first or latest data sets had also been published earlier [McKauge et al 1981, Suzuki et al 1991].

Studies of the correlation between dose and serum level have been contradictory since both linear and non-linear had been reported(table 7-7). The present results are in-line with the latter observation. The current results are also similar to a prospective study on Malaysian patients by Ismail & Rahman 1993 where no such relationship was reported. This observation is in fact contradicting the theory that at steady-state conditions, the relationship between dose and steady-state serum level is linear [Wagner 1965]. This current result clearly validates that carbamazepine is not among the group of drugs characteristic of this behaviour.

Table 7-7: Studies that reported either linear or non-linear relationship between dose and serum level of carbamazepine

Final conclusion -Authors	**Therapy**	**Sample size**	**Patient's type**	**Dose given**	**Ethnicity**
I. Non-linear relationship					
-Rane et al 1976	Polytherapy	20	Paediatric patients	mg/kg.day	-
-Eichelbaum et al 1976	Polytherapy	13	Not defined	mg/kg.day	-
-Strandjord & Johannessen 1977	Mixed	28	Mixed age patients	mg/kg.day	-
-Mihaly et al 1977	Not mentioned	35	Mixed age patients	mg/day	-
-Schain et al 1977	Mixed	37	Paediatric patients	mg/kg.day	-
-Sato et al 1979	Monotherapy	27	Paediatric patients	mg/kg.day	-
-Pynnonen et al 1980	Not mentioned	35	Adult patients	mg/kg.day	-
-Strandjord & Johannessen 1977	Mixed	62	Mixed age patients	mg/kg.day	-
-McKauge et al 1981*	Mixed	295	Mixed age epileptics	mg/kg.day	-
-Bourgeois & Wad 1984	Polytherapy	33	Mixed age epileptics	ma/kg.day	-
-Elyas et al 1986	Mixed	68	Paediatric patients	mg/kg.day	-
-Belle & Friel 1986	Mixed	24	Adult patients	mg/kg.day	-
-Tomson et al 1989	Mixed	13	Adult patients	mg/day	-
-Irfan et al 1989*	Monotherapy	183	Mixed age patients	mg/m.sq	-
-Wang et al 1990	Mixed	135	Mixed age epileptics	mg/kg.day	-
-Ichikou et al 1990	Mixed	27	Adult patients	mg/kg.day	-
-Wang et al 1990	Mixed	135	Mixed patients	mg/kg.day	-
-Suzuki et al 1991*	Polytherapy	107	Paediatric patients	mg/kg.day	-
-Yukawa et al 1992*	Polytherapy	370	Paediatric patients	mg/kg.day	Japanese
-Ismail & Rahman 1993	Monotherapy	14	Mixed age epileptics	mg/day	Malaysian (mostly Malays)
-Hua Liu & Delgado 1994	Not mentioned	55	Paediatric patients	mg/kg.day	-
II. Linear relationship					
-Cereghino et al 1973	Polytherapy	8	Adult patients	mg/day	-
-Rane et al 1976	Monotherapy	23	Paediatric patients	mg/kg.day	-
-Perucca et al 1978	Mixed	80	Adult patients	mg/kg.day	-
-Huf & Schain 1980	Mixed	61	Paediatric patients	mg/kg.day	-
-Rylance & Moreland 1981#	Mixed	35	Paediatric patients	mg/m^2	-
-Kumps AH 1981	Mixed	24	Adult epileptics	mg/kg.day	-
-Kumps AH 1981	Mixed	26	Paediatric epileptics	mg/kg.day	-
-Bourgeois & Wad 1984	Monotherapy	14	Adult patients	ma/kg.day	-
-Hartley et al 1990	Monotherapy	21	Paediatric patients	mg/kg.day	-
-Larkin et al 1991*	Mixed	106	Not defined	mg/day	-

* : Retrospective studies, otherwise prospective, # : Measurement by saliva, if not plasma/serum

This non-linear relationship is linked to the autoinduction properties of carbamazepine [Tomson et al 1989, Bernus et al 1996]. Autoinduction in carbamazepine was originally observed by Eichelbaum et al (1974) as they reported the half-life was shorter and steady-state serum level lower in multiple dose compared to single dose patients. They further commented that carbamazepine induces its own metabolism during repeated administration and this characteristic is called autoinduction.

Autoinduction in carbamazepine leads to the reputed dose-dependent kinetics of the drug. Dose-dependent pharmacokinetics is the phenomenon where a high increase in dose lead to small increase in serum level. The small increase in serum level is directly proportional to increased clearance where relationship between dose and clearance had been reported to be linear [Kudriakova et al 1992]. Tomson et al (1989) relates this observation by the higher ratios of carbamazepine metabolite to total carbamazepine, i.e. trans-CBZ -diol/CBZ ratios. They further explained the increased in ratio led to lower serum concentration and this is characteristic for the dose-dependent pharmacokinetic relationship.

Bernus et al (1996) also proposed the possibility of the dose-dependent relation being explained by incomplete oral bioavailability. Since bioavailability has been reported to be both affected [Levy et al 1975] and unaffected [Tedeshi et al 1981] by food, the extent to which it affected the present study is unknown. Also, since the studied patient groups were of different ethnic groups, the possibility of differences in bioavailability cannot be discounted. This is clearly important in this study where the bioavailability factor(F) used was equated to one in all ethnic groups. Bioavailability factor might contribute to interindividual and possibly interethnic variation during the estimation of apparent clearance which can lead to the final predicted serum concentration being lower than it should be.

The better correlation of dose in mg/day than mg/kg to serum concentration observed in the present study has similarly been reported by Bernus et al (1996). Results for paediatric patients showed that the correlation values for dose in mg/day had a better overall relationship (r_{adj} square = 32.4%) than dose in mg/kg (r_{adj} square = 16.1%). Results from adult patients however similarly showed higher correlation values for dose in mg/day (r_{adj} square = 36.0%) than mg/kg (r_{adj} square = 34.6%)

but the differences were not entirely distinctive. These findings verify the present recommendation that carbamazepine dosing guidelines [BNF 1996] is based on therapeutic response rather than body weight. Bernus et al (1996) contributed the reasons for carbamazepine non-linearity behavior reported in earlier studies to the common expectation that allowance for body weight would provide better measure of serum concentration.

The results from this study are in accord with the many earlier authors that the relationship between dose and clearance is linear where an increase in dose lead to a proportionate increase in clearance [Eadie & Tyrer 1980, Meinardi 1975, Rambeck et al 1987, Schneider 1975]. The current result nevertheless contradicts prospective studies by Sanchez et al (1986) and Ismail & Rahman (1993). Sanchez et al (1986) reported this relationship is true for children but not for adult but noted problems of compliance might have affected their findings. Ismail & Rahman (1993) however reported no such relationship. Their anomalous finding may be explained by a population size of only 14 patients and a limited dose range between 400 to 600 mg/day which might restrict the chance of demonstrating a similar relationship [Hartley et al 1990, Perucca et al 1980].

The results showed that the difference in clearance of paediatric patients significantly faster than adults and is expected. Similar findings on the rate of elimination of paediatric patients has long been reported to be considerably higher than adults by Rey et al (1979), Moreland et al (1982), Pynnonen et al (1977) and Rylance et al (1979).

The impact of inter-ethnic differences on the linear relationship between dose and clearance were found to be statistically insignificant in both adult and paediatric patients of Malay, Chinese and Indian origin. These results showed that the effect of differences in age, weight, dose and serum level in either adult or paediatric patients have no significant influence on inter-ethnic differences.

Comparing the final regression equation(table 7-7) for the paediatric Malaysian population to Black Africans reported by Summers & Summers (1989) suggested that inter-ethnic differences were clearly absent. As for adult patients, no confounding differences were also observed between Malaysians, Australians [Bernus et al 1996], and Germans [Kudriakova et al 1992] population. These findings

decisively showed that inter-ethnic differences have no influence on the dose-dependent pharmacokinetics of carbamazepine in chronically treated patients.

Table 7-7: Comparison of clearance(CL) and dose(D) relationship with publish studies

Studies	Correlation equation	r^2	t-ratio	SE-value	variance -slope	Homogeneity test-p value
1. Paediatric						
-current study	CL=0.02+0.006D(mg/kg)	0.72	18.80	0.1128	0.0127	
-Summers & Summers 1989	CL=0.03+ 0.002D(mg/kg)	0.49	9.19	0.0184	0.0003	
Pooled						0.49
2(a). Adults						
-current study	CL=29.5 + 0.06D(mg/day)	0.53	21.87	1.31	1.72	
-Bernus et al 1996	CL=28.98 + 7.58D(mg/day)	0.55	4.28	32.45	1053.18	
-Kudriakova et al 1992	CL=25.2 + 0.07D(mg/day)	0.90	28.02	1.82	3.32	
Pooled						0.49

The predicted curvilinear dose and serum level relationship observed in this study was similar to that concluded by Kudriakova et al (1992) and Summers & Summers (1989). A similar curvilinear relationship was reported by Yukawa et al (1992) where they derived similar relationship from the significantly related dose/serum level ratios to dose. However, the presence of wide scatter signify a considerably large inter-individual variation.

This relationship is highly likely from the many reports of carbamazepine dose-dependent kinetics. In current perspective, the non-linear relationship has been widely used and accepted in studies to determine carbamazepine population pharmacokinetics using retrospective population pharmacokinetic data.

7.5 Limitations of study

Difficulties in the current retrospective study have been discussed in chapter 5. However, the present study has two limitations which may affect the overall findings.

The first is the factor for bioavailability(F) which was assumed to equal to one for estimating apparent clearance. Although, the estimated bioavailability factor by Ismail & Rahman (1993) had showed that its value is close to one, no studies have

yet been initiated to accurately measure population values specific for Malaysian multi-ethnic epileptic population. Therapeutic bioequivalency studies of carbamazepine have been shown to vary between products [Bhatia et al 1988] while Jumao et al (1989), Hartley et al (1990) and Oles et al (1992) had all shown no significant differences. Comparative studies between chewable [Patsalos et al 1990, Cornaggia et al 1993], slow release [Reunanen et al 1990] and conventional carbamazepine similarly showed no significant differences in bioequivalence although the latter had been reported to be less efficacious and have more frequent occurrences of side-effects [Reunanen et al 1990]. Carbamazepine bioavailibility was also reported to be increased by food [Levy et al 1975] and this could also contribute to variation in serum concentration.

Secondly, the minimum period of therapy is linked to the time of autoinduction. Time for autoinduction to stabilise has been noted to be between 21 to 30 days [Browne et al 1989, Mikati et al 19, Bertilsson 1980] and 30 days was assumed for the minimum period of therapy during selection of patients in Malaysian patients. However, reports on Chinese [Lin et al 1992] and Koreans [Yoon et al 1996] healthy volunteers time for autoinduction of approximately 46 days showed that there is the possibility of inter-ethnic differences in carbamazepine rate of metabolism. Critically, inter-ethnic differences in the time for autoinduction to complete could well be present in Malays, Chinese and Indians. Thus, 30 days might not be sufficient for all ethnic groups.

7.6 Conclusion

The exact relationship between dose and serum level for carbamazepine has yet to be confirmed although it has been 25 years after the earliest report in 1973 was first published. Linear relationship were mostly observed in the earlier year of 1970 and 1980 while lately most of the published reports tend to favour the non-linear relationship. The present study showed a weak and a statistically insignificant relationship between dose and serum level. High inter-individual variation was also observed in all ethnic groups with Indian and Chinese patients displaying values much higher than their Malay counterparts.

Large population-based studies [Yukawa et al 1992, Summers & Summers 1989] and evidence of dose-dependent pharmacokinetics of carbamazepine [Kumps AH 1981] has strengthened the latter claim and reduce the former claim of linearity now believed to be due to study faults or inappropriate study design [Bernus et al 1996]. However, authors of reported linear relationship [Perucca et al 1978, Huf & Schain 1980] had similarly found the presence of a wide scatter around the regression line to be related to high inter-individual variations. They also mentioned that high inter-individual variation makes estimating serum level directly from the prescribed dose unpredictable.

The true nature of the non-linear relationship is still unconfirmed. To date, reports by Eadie & Tyrer (1980), Rambeck et al (1987), Summers & Summers (1989), and Kudriakova et al (1992) had all reported the curvilinear relationship between dose and serum level but no consensus has yet been reached. The result of this study nevertheless substantiated the possibility of the curvilinear theory although further investigation may need to be initiated to confirm this finding.

Evidence of inter-ethnic differences in the dose-dependent kinetics of carbamazepine among Malay, Chinese and Indian epileptic population in Malaysia was found to be statistically unlikely. Comparison with published reports on Blacks, Australian and German patients on chronic carbamazepine therapy showed similar results.

The possibility of ethnic differences may however be detected by investigating population pharmacokinetic parameters such as half-lifes(related to time of autoinduction), clearance etc, and intra and interindividual differences among patient groups. This finding may prove conclusive since reports of single and multi-dose studies has shown significant inter-ethnic differences in the half-lifes of carbamazepine between Orientals (Korean and Chinese) [Yoon et al 1996 and Lin et al 1991] and Caucasian.

Finally, the present study presented the first ever relationship between dose and serum level in both paediatric and adult Malaysian epileptics. This formula might proved useful for estimating serum level especially in hospitals where therapeutic drug monitoring services are not provided. However, it should be stressed that this formula should act as a basic guideline in carbamazepine therapy and its practical use may

need to be restricted to determining suspected non-compliance and toxicity only. Clinical judgment is still crucial for optimum therapeutic outcome because of well-known high inter-individual variation in carbamazepine kinetics.

Chapter 8:

Valproic acid: Steady-state pharmacokinetics in a multi-ethnic epileptic population

8.1 Introduction

Valproic acid or the salt form sodium valproate(sodium dipropylacetate) is currently used as an anticonvulsant agent in the treatment of generalized tonic-clonic and absence seizures [Chadwick DW 1987]. Although, this drug has been used for more than 20 years after it was first discovered in 1963, the relationship between valproic acid dose and serum concentration has yet to be defined.

Monitoring of total serum level of valproic acid is complicated by valproic acid exhibiting the phenomenon called concentration-dependent serum protein binding [Bowdle et al 1980]. This is a condition where increase of serum level is dependent on serum albumin binding sites. At saturation of binding sites, serum levels of unbound valproic acid fraction will increase disproportionately, which consequently leads to increase in the rate of elimination but decrease in total serum level. Therefore, as total serum level increases, theoretically the relationship between dose and serum levels becomes curvilinear, that is, incremental increase in dose produces less elevation of total serum valproic acid concentrations [Levy et al 1986].

Secondly, valproic acid is known to display high interindividual variation, which leads to the consistently poor relationship between dose and serum levels among patients [Cloyd et al 1983, Dodson & Tasch 1981, Bruni et al 1978]. This has made dosage determination and adjustment complicated.

Poor correlation between dose and serum level has been attributed to differences in valproic acid clearance. Evidence which suggests clearance changes with age and/or polytherapy have been reported to be responsible for interindividual variation [Levy RH 1984]. All these factors are however linked to the way in which valproic acid is metabolised and eliminated.

Valproic acid is primarily metabolised and eliminated by the liver [Levy et al 1986]. Since liver function changes with maturity(age), steady-state serum levels could be expected to vary among individuals with different metabolic capabilities. This is clearly

demonstrated where clearance of adults has been showed to be higher than children [Dodson & Tasch 1981, Cloyd et al 1983]. The inconsistent relationship between dose and serum levels observed in several studies might have been due to the inclusion of both paediatric and adult age groups.

Clearance of valproic acid has been shown to be higher when taken with other antipeileptic drugs [Henriksen & Johannessen 1982]. An increase in enzyme induction activities induced by concomitant antiepileptic drugs leads to an increased clearance of valproic acid [Sackellares et al 1981]. This factor may also contribute to the lack of correlation between dose and steady-state serum level of valproic acid.

Enzymes responsible for the hepatic metabolism of antiepileptic drugs have been known to be genetically controlled [Kalow et al 1982]. By contrast to valproic acid, differences in inter-ethnic rates of metabolism have been published for phenytoin [Grasela et al 1983] and several studies [Chan et al 1990, Rui et al 1995] have reported on its ethnic-specific population pharmacokinetics. Evidence on carbamazepine has yet to be established although differences have been reported [Yoon et al 1996, Lin et al 1991]. Nevertheless, comparative studies examining for inter-ethnic differences for valproic acid has been reported by Botha et al (1995) and their results showed that valproic acid mean clearances in South Africa Black and Indian paediatric patients were within the normal quoted range. However, more studies of this nature are needed to disapprove this assumption of inter-ethnic differences in valproic acid metabolism.

Based on above information concerning valproic acid, this study aims to determine the relationship between valproic acid dose and serum concentration relationship among both paediatric and adult patients groups of Malay, Chinese and Indian ethnic origin. Differences in treatment strategy(monotherapy and polytherapy) and interindividual variation will be the other main focus of study. Essentially, this study hope to establish evidence to prove that there are inter-ethnic differences in valproic acid metabolism.

8.2 Methods

Patient data were obtained retrospectively using the method described in detail in chapter in chapter 5. A total of 473 steady-state dose and serum data pairs were selected

utilising the randomisation strategy described in chapter 5. 236 adult and 237 paediatric epileptic patients were included in the study.

Patients were divided into groups according to type of therapy(monotherapy or polytherapy), age and ethnicity. The ethnic groups studied were Malay, Chinese and Indian and were subdivided further according to age. Patients were categorised as paediatric or adult based on the age limit of 18 years old. The clinical characteristics of the patients pertinent to the study are presented in table 8-1 and 8-2.

Table 8.1: Summary data of adult patients

Characteristic	Malays	Chinese	Indians
No. of patients	88	75	72
Ratio of male/female	45:43	32:43	38:34
Age(yr)			
Mean,sd	33.60,11.00	33.15,12.07	30.37,11.24
Range	18.00-64.00	18.00-69.00	18.00-54.00
Weight			
Mean,sd	56.85,10.93	56.63,8.51	56.01,11.55
Range	34.00-80.00	37.00-74.20	31.00-89.00
Disease duration(yr)			
Mean,sd	12.97,9.60	13.87,8.91	13.87,10.88
Range	1.00-38.00	1.00-45.00	1.00-40.00
Epilepsy Types			
Generalised	65	56	64
Partial	11	12	11
Others	12	7	12
Therapy(M:P)	32:56	16:59	24:46
Dose(mg/day)			
Mean, sd	879.50,421.90	813.30,359.90	750.00,352.10
Range	200-2000	200-1900	200-1600
Dose(mg/kg)			
Mean,sd	16.47,9.77	14.71,7.14	14.18,8.20
Range	3.33-51.28	3.28,41.86	3.03-38.46
Css(mg/L)			
Mean,sd	43.16,20.40	37.25,17.85	41.30,17.67
Range	3.33-125.34	7.71-82.11	3.60-74.79
CL(L/kg.day)			
Mean,sd	0.48,0.49	0.46,0.33	0.39.0.30
Range	0.15-3.03	0.19-2.67	0.10-2.18

Abbreviation: M- monotherapy, P- polytherapy

Non-compliant patients were determined from patient notes and plasma concentration request forms and were excluded. Details pertaining to each dose and serum concentration selected were checked from patient notes for confirmation. The serum level was measured by the fluorescence polarization immunoassay(FPIA) method. Patients were only included if they were on valproic acid therapy for a minimum period of 30 days.

The relationship between dose and serum level was determined by linear regression analysis and Pearsons correlation coefficient and the significance of correlation was determined using Student's *t* test [Daniel WW 1987]. To ease calculation, MINITAB version 10 were used for all calculation. A P value less than or equal 0.05 was considered significant.

Table 8.2: Summary data of paediatric patients

Characteristic	Malays	Chinese	Indian
No. of patients	116	64	60
Ratio of male/female	65:51	36:28	35:25
Age(yr)			
Mean,sd	8.65,4.47	8.18,4.00	10.12,4.36
Range	1.00-17.00	4-17.00	5.00-17.00
Weight			
Mean,sd	27.22,14.07	27.09,13.30	30.35,12.91
Range	5.10-58.00	16.00-80.00	10.30-57.00
Disease duration(yr)			
Mean,sd	5.63,3.87	6.29,3.95	5.91,3.51
Range	0.50-16.00	0.50-14.00	2.00-12.00
Epilepsy Types			
Generalised	84	36	36
Partial	13	11	21
Others	19	17	3
Therapy(M:P)		48:16	
Dose(mg/kg)			
Mean,sd	23.83,20.54	23.86,11.74	21.29,12.54
Range			
Dose(mg/day)			
Mean, sd	545.10,362.40	616.10,353.10	629.20,414.30
Range	100-1500	90-2000	150-2000
Css(mg/L)			
Mean,sd	56.62,31.61	67.13,30.78	65.12,38.87
Range	4.06-197.19	10.69-141.56	8.71-174.01
CL(L/kg.day)			
Mean,sd	0.51,0.46	0.40,0.25	0.44,0.36
Range	0.11-3.43	0.13-1.50	0.09-2.39

Abbreviation: M-monotherapy, P-polytherapy

The variables used for analysis was dose, serum level, and apparent clearance. Doses were expressed in either milligram per day(mg/day) or per kilogram(mg/kg), serum levels in milligram per liters(mg/L) and clearance in Liters per kg per day(L/kg.day). The formula by Wagner (1965) and is described in chapter 1, section 1.5.5, was used to calculate apparent clearance,

$$Clearance(CLij) = \frac{F.Dij}{Css_{ij}.\tau} \qquad \textit{(equation 7-1)}$$

where Css_{ij} is the steady state blood concentration, D_{ij} is the dose, τ is the length of the dosage interval, F is the fraction of each dose which is absorbed(bioavailibility factor).

Plotting *Css* and D_{ij} would be a straight line with the y intercept of 0 and the assumption that the other variables remains constant. *F* has been reported to be between 0.85 to 1 in various oral dosage forms [Perucca et al 1978, Johannessen & Henriksen 1980]. For simplicity, *F* was taken as equal to 1. The formula assumes that at steady state (at equilibrium) the ratio of change is proportionate to the ratio of change of serum concentrations. Clearance is measured in liters per kg.day (L/kg.day). Correlation of clearance and dose were evaluated by linear regression [Daniel 1987].

The method proposed by Greenland 1971 was used to determine for inter-ethnic differences. Detail outlining the formulas were described in chapter 3, section 3.3.3(b). Differences in age, sex, disease duration and epilepsy types in each group were evaluated by one way analysis of variance(ANOVA). Coefficient of variation(CV) for interindividual variation was calculated using the following formula: CV(%) = (SD ÷ Mean) x 100, where 'SD'denotes standard deviation.

8.3 Results

Analysis of variance of in adult patients found that there were significant differences among adult patients for the variables such as age, weight, length of disease years, dose and serum concentration. Significant differences between ethnic groups were found for serum concentration($p<0.05$) in paediatric patients.(Table 8-3).

Table 8-3: Analysis of variances of variables

Variables	Malay (mean,sd)	Chinese (mean,sd)	Indian (mean,sd)	p-value (mean,sd)
1. Adult-n				
-Age(years)	33.60,11.00	33.15,12.07	30.37,11.24	0.171
-Weight(kg)	56.85,10.93	56.63,8.51	56.01,11.55	0.874
-Length of disease years(years)	12.97,9.60	13.88,8.91	13.87,10.88	0.792
-Dose(mg/day)	879.50,421.90	813.30,359.90	750.00,352.10	0.104
-Serum concentration(mg/L)	43.16,20.40	37.25,17.85	41.30,17.67	0.131
2. Children-n				
-Age(years)	8.65,4.47	8.18,4.00	10.12,4.36	0.032
-Weight(kg)	27.22,14.07	27.09,13.30	30.35,12.91	0.292
-Length of disease years(years)	5.63,3.87	6.30,3.95	5.91,3.51	0.530
-Dose(mg/day)	545.10,362.40	616.10,353.10	629.20,414.30	0.274
-Serum concentration(mg/L)	56.62,31.61	67.13,30.78	65.12,38.87	0.084

8.3.1 Relationship between dose and serum level

Plots of dose and serum level in paediatric and adult patients(irrespective of therapy) showed that a rather weak linear relationship existed for both dose in mg/day or mg/kg(figure 8-1 to 8-4). The corresponding correlation coefficient(r_{adj}) values for dose in mg/day and mg/kg in paediatric and adult patients were 0.47($p < 0.01$) and 0.17($p < 0.01$) and 0.32($p < 0.01$) and 0.29($p < 0.01$) respectively.

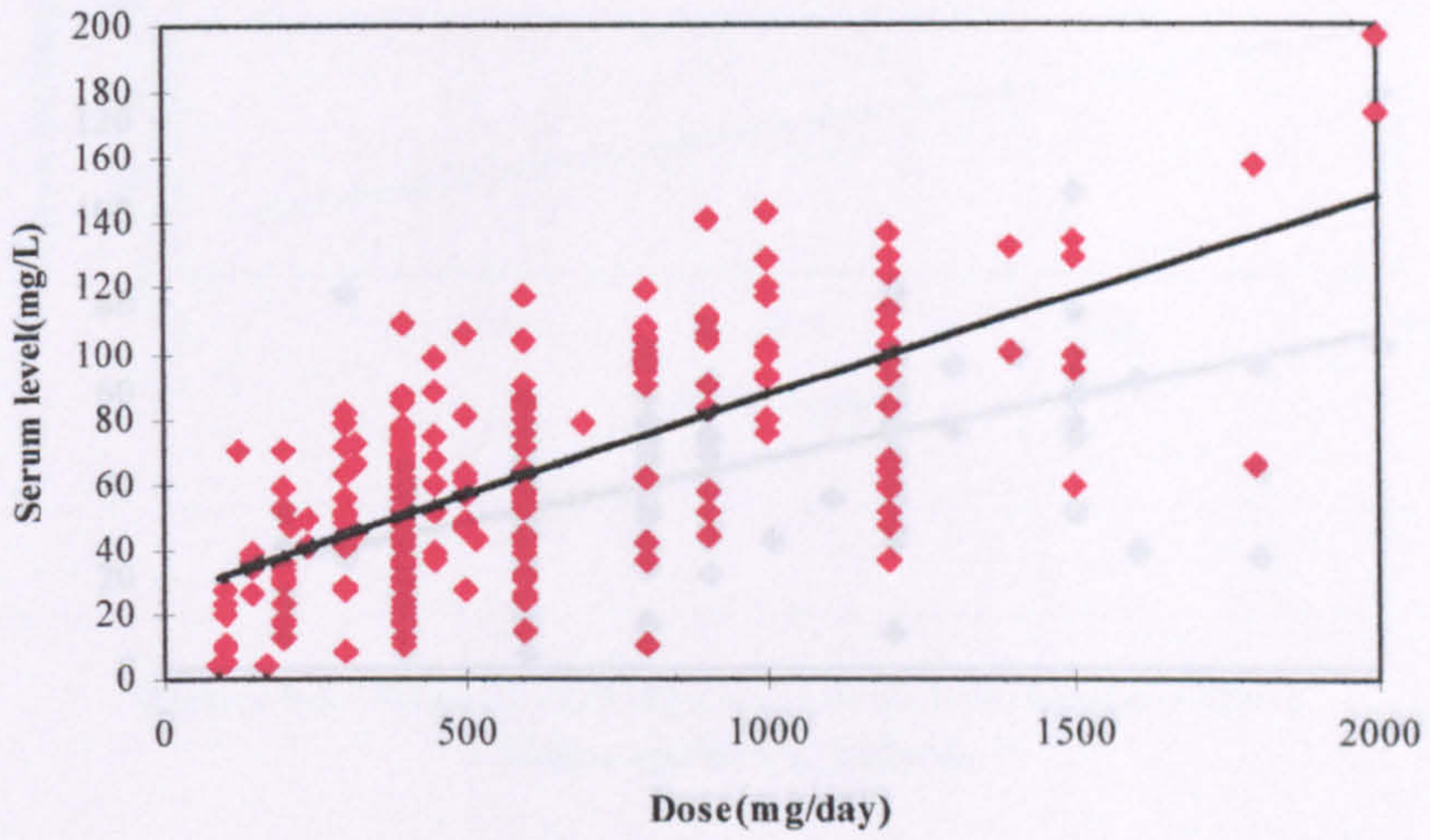
Table 8-4: Dose and serum level(SL) relationship in paediatric and adult patients

Predictors	Malays	Chinese	Indians
I. Paediatric			
a) Regression equation (dose,mg/kg)	SL=42.5 + 0.59Dose	SL=33.7 + 1.40Dose	SL=28.2 + 1.73 Dose
R-square(adj)	14.2%	27.2%	31.3%
F value	20.02	24.57	26.45
p-value	0.00	0.00	0.00
C.V(serum level/dose)	61.74	48.41	65.56
b) Regression equation (dose,mg/day)	SL=27.3 + 0.0538Dose	SL=30.6 + 0.0593Dose	SL=19.1 + 0.0732Dose
R-square(adj)	38.1%	45.5%	60.2%
F-value	70.13	53.50	90.24
p-value	0.00	0.00	0.00
C.V(serum level/dose)	57.85	45.91	54.66
II. Adult			
a) Regression equation (dose,mg/kg)	SL=24.4+ 1.14Dose	SL=13.6 + 1.61 Dose	SL=25.7 + 1.04 Dose
R-square(adj)	29.0%	40.7%	23.5%
F-square	36.60	51.76	22.81
p-value	0.00	0.00	0.00
C.V(serum level/dose)	50.45	43.03	53.09
b) Regression equation (dose,mg/day)	SL=19.4 +0.0271Dose	SL=11.8 + 0.0313Dose	SL=18.5 + 0.0293Dose
R-square(adj)	30.5%	38.9%	35.1%
F-value	39.19	48.19	39.31
p-value	0.00	0.00	0.00
C.V(serum level/dose)	45.09	42.87	49.13

Abbreviation: C.V, coefficient of variation.

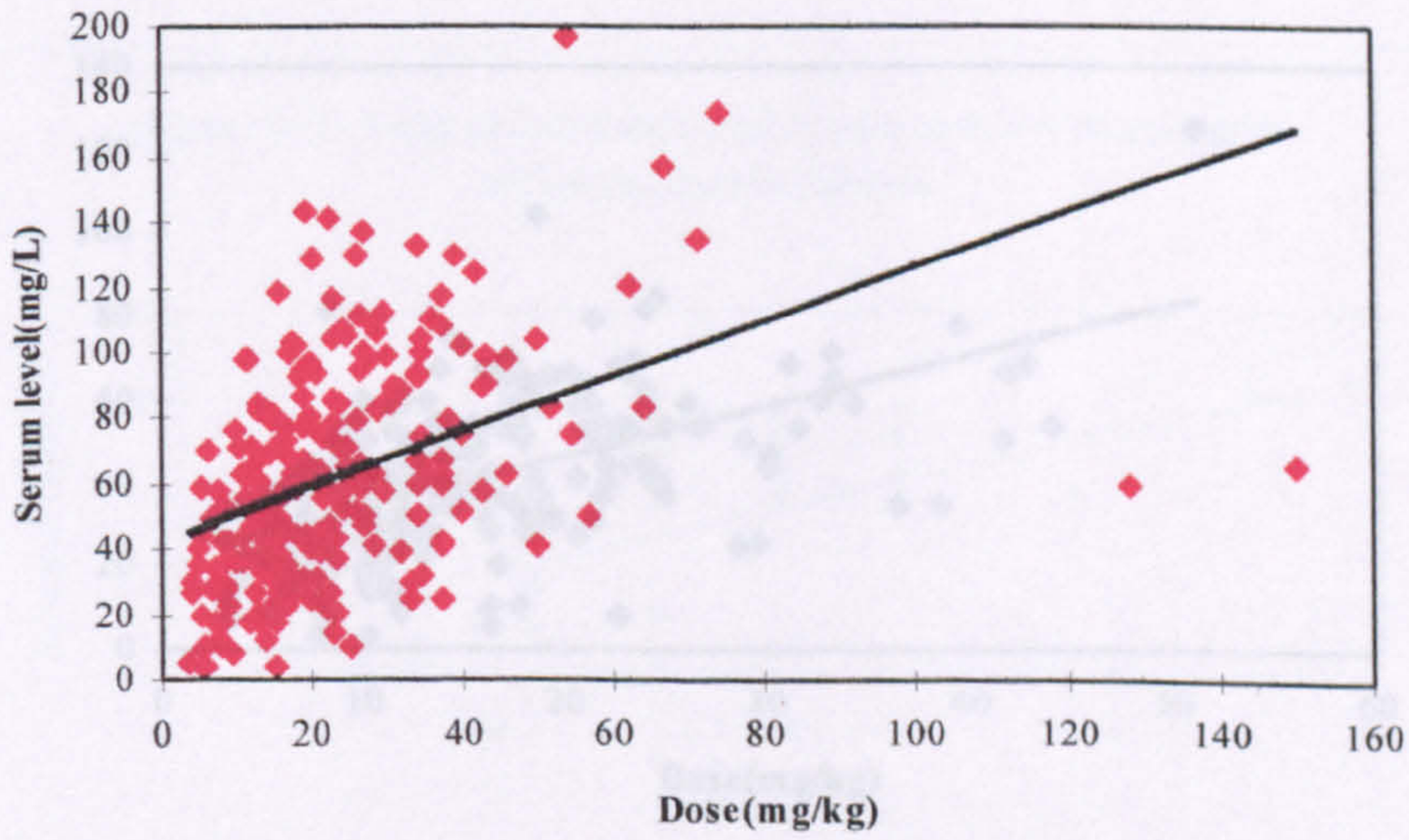
Figures 8-1 to 8-4 are also characterised by a wide scatter of data points about the regression line which is an indicator for high interindividual variation. The mean serum level:dose ratio(sd) for dose given in mg/day and mg/kg in paediatric patients was 0.1238(0.0665) and 3.213(1.917) and the calculated coefficient of variation(CV) was 53.71% and 61.38%. The corresponding serum level:dose ratios for adults were 0.0557(0,0294) and 3.133(1.625). The coefficient of variation for adults were 52.78% and 51.87%. These high values of coefficient of variation noted in both paediatric and

Figure 8-1: Valproic acid dose and serum level relationship in paediatric patients



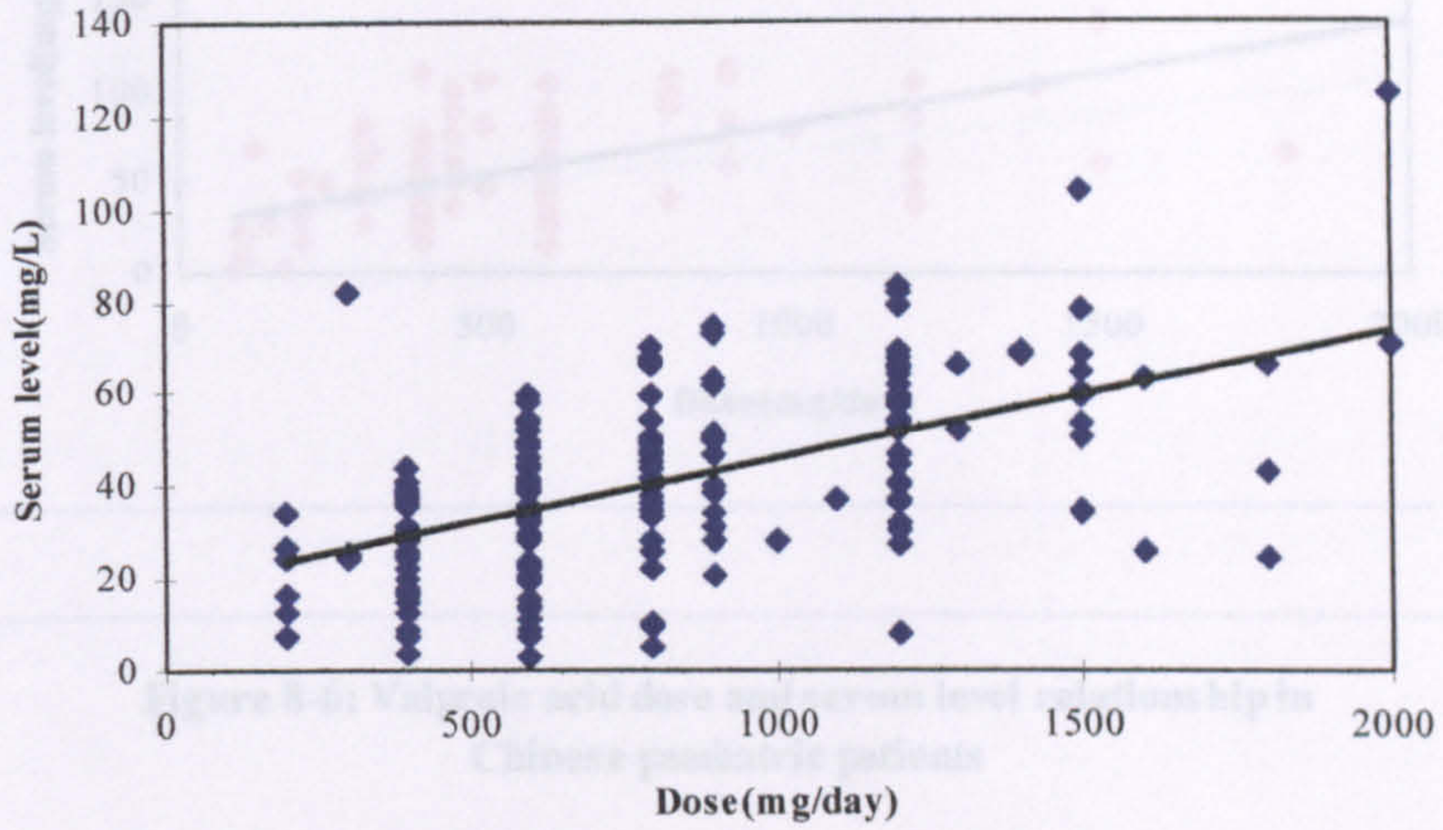
Regression line : Y = 25.45 + 0.06X (R-squared =0.473)

Figure 8-2: Valproic acid dose and serum level relationship in paediatric patients



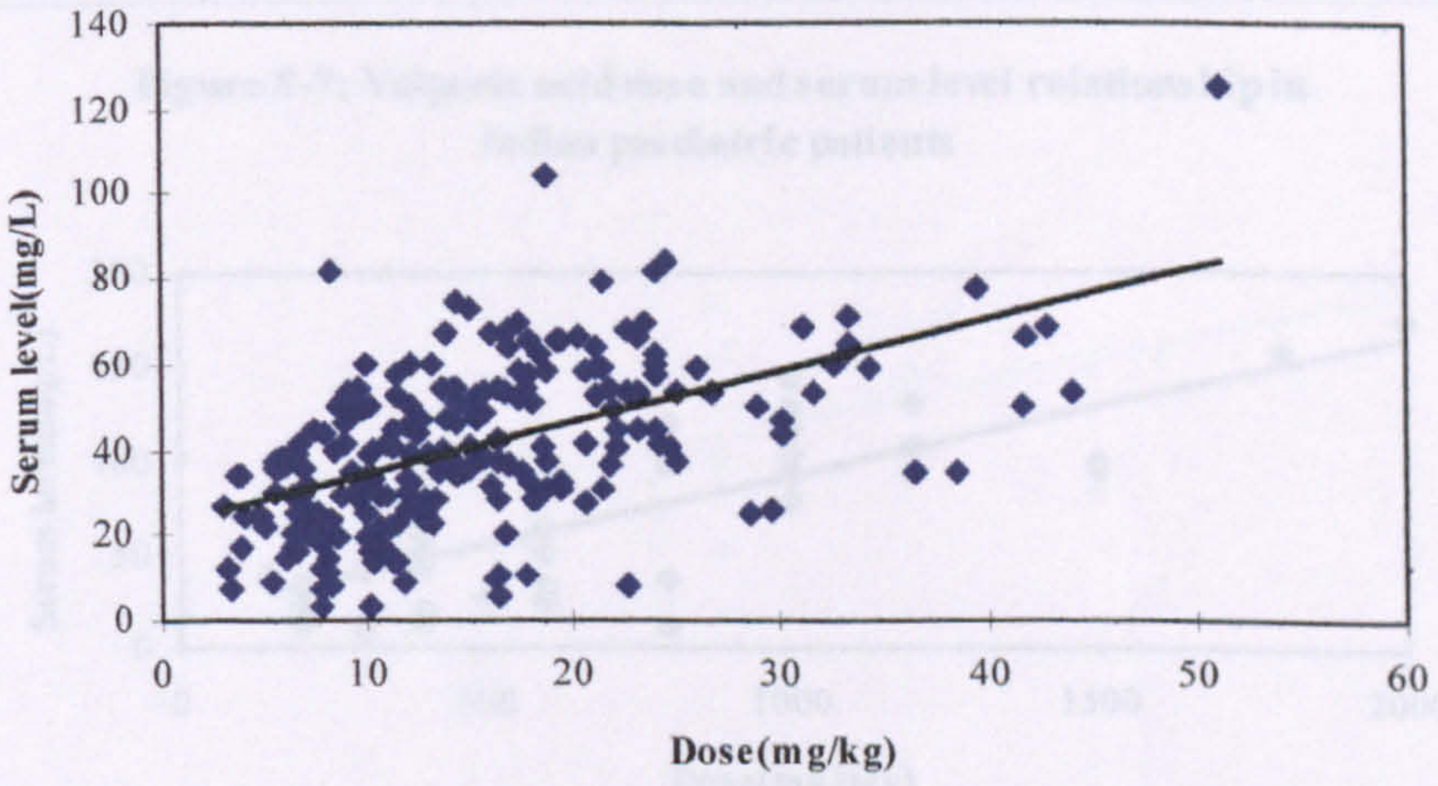
Regression line : Y = 41.67 + 0.85X (R-squared = 0.176)

Figure 8-3: Valproic acid dose and serum level relationship in adult patients



Regression line : Y = 17.96 + 0.03X (R-squared = 0.320)

Figure 8-4: Valproic acid dose and serum level relationship in adult patients



Regression line : Y = 22.46 + 1.20X (R-squared = 0.295)

Figure 8-5: Valproic acid dose and serum level relationship in Malay paediatric patients

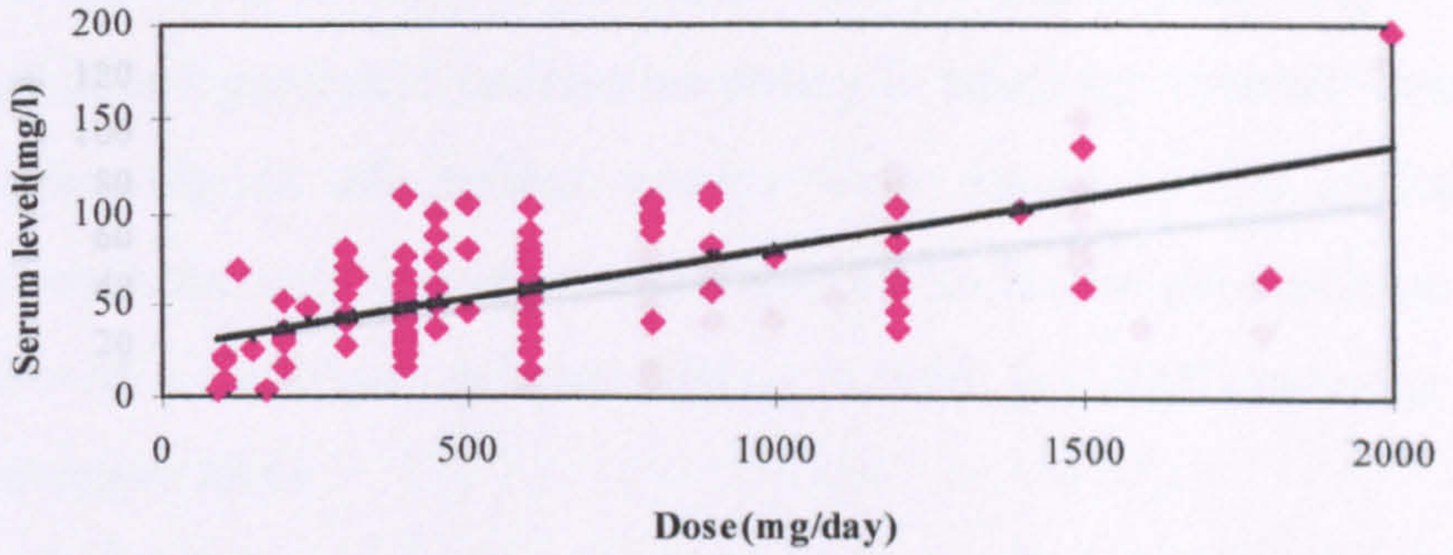


Figure 8-6: Valproic acid dose and serum level relationship in Chinese paediatric patients

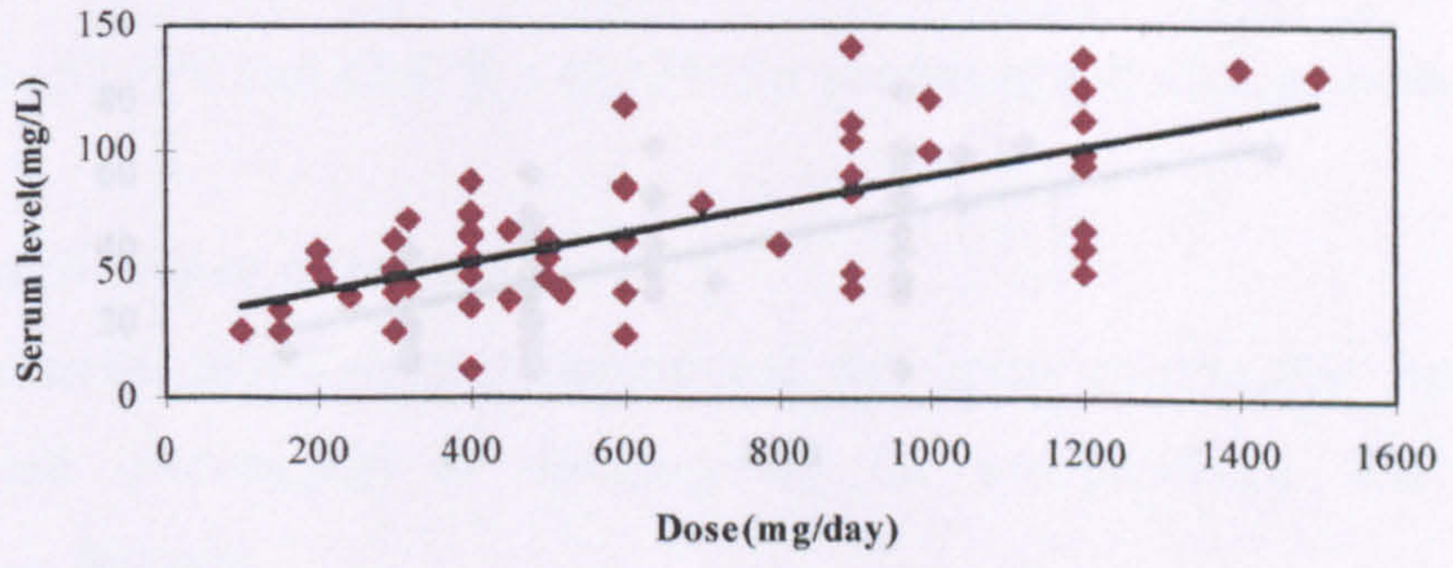


Figure 8-7: Valproic acid dose and serum level relationship in Indian paediatric patients

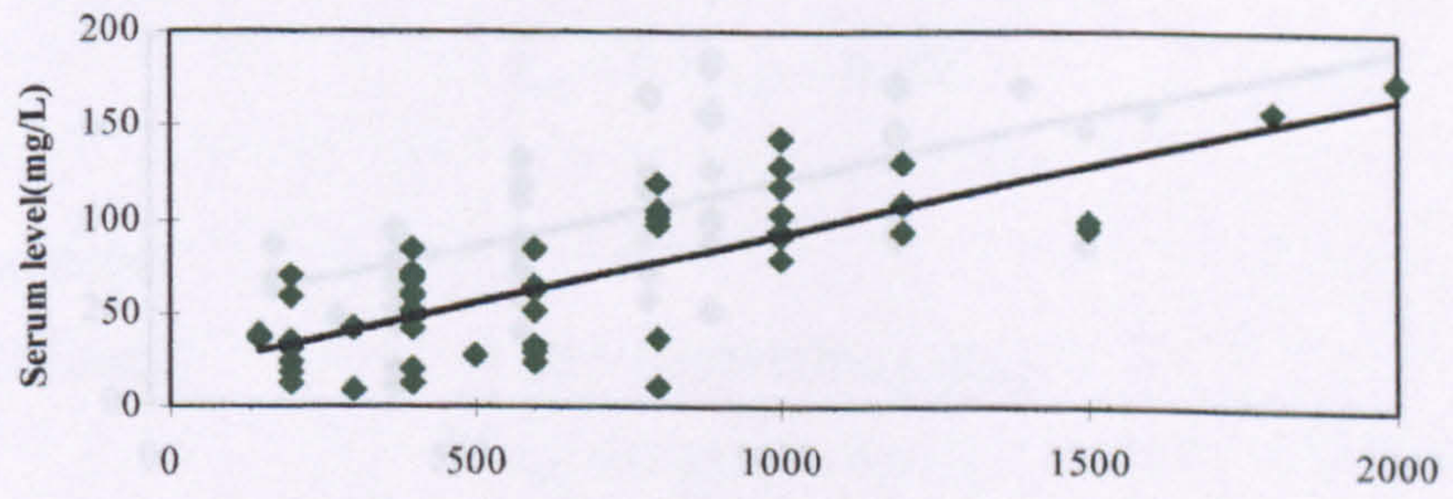


Figure 8-8: Valproic acid dose and serum level relationship in adult Malay patients

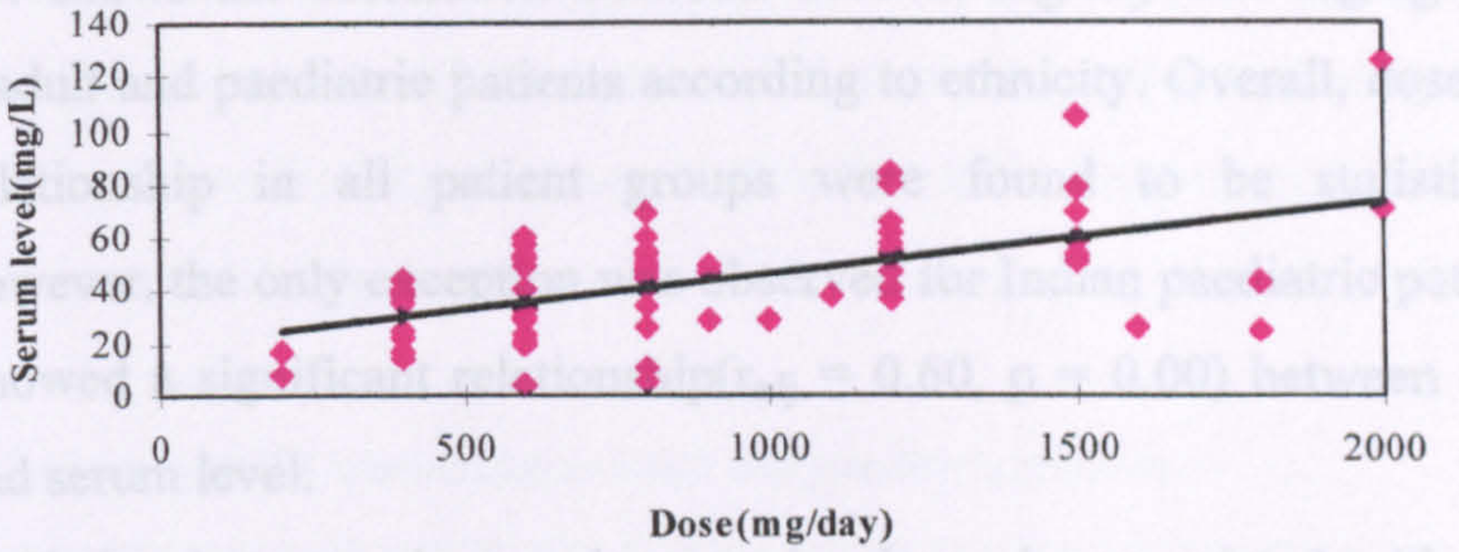


Figure 8-9: Valproic acid dose and serum level relationship in Chinese adult patients

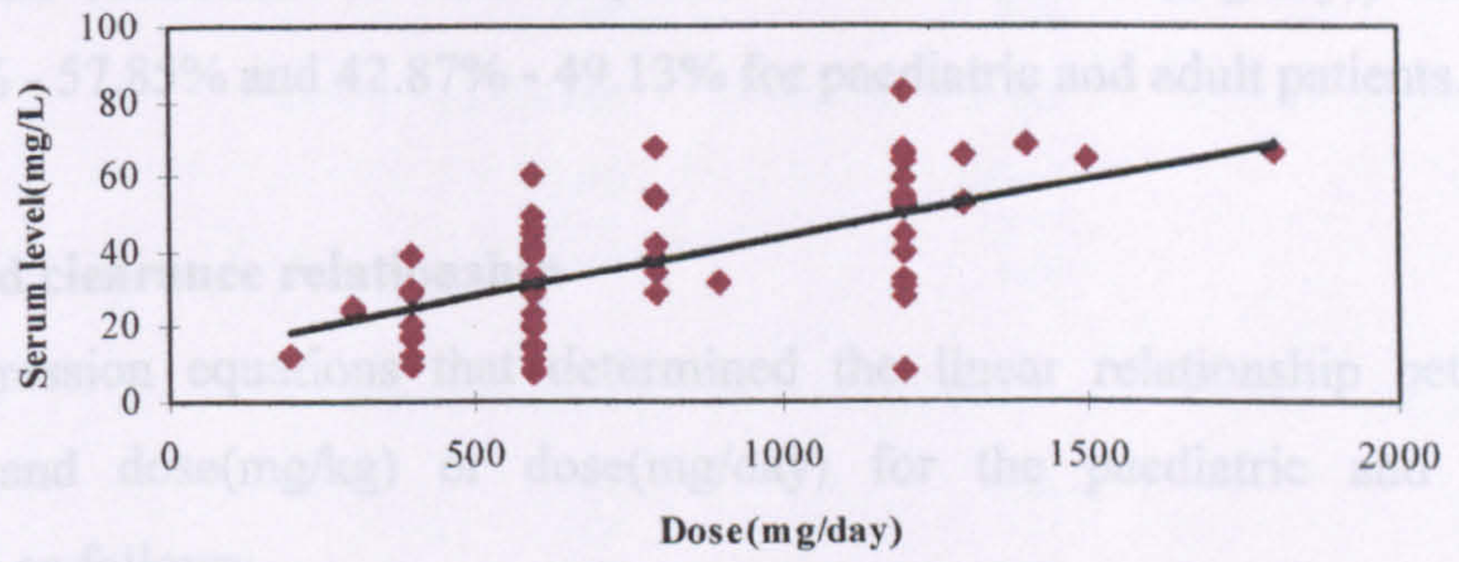
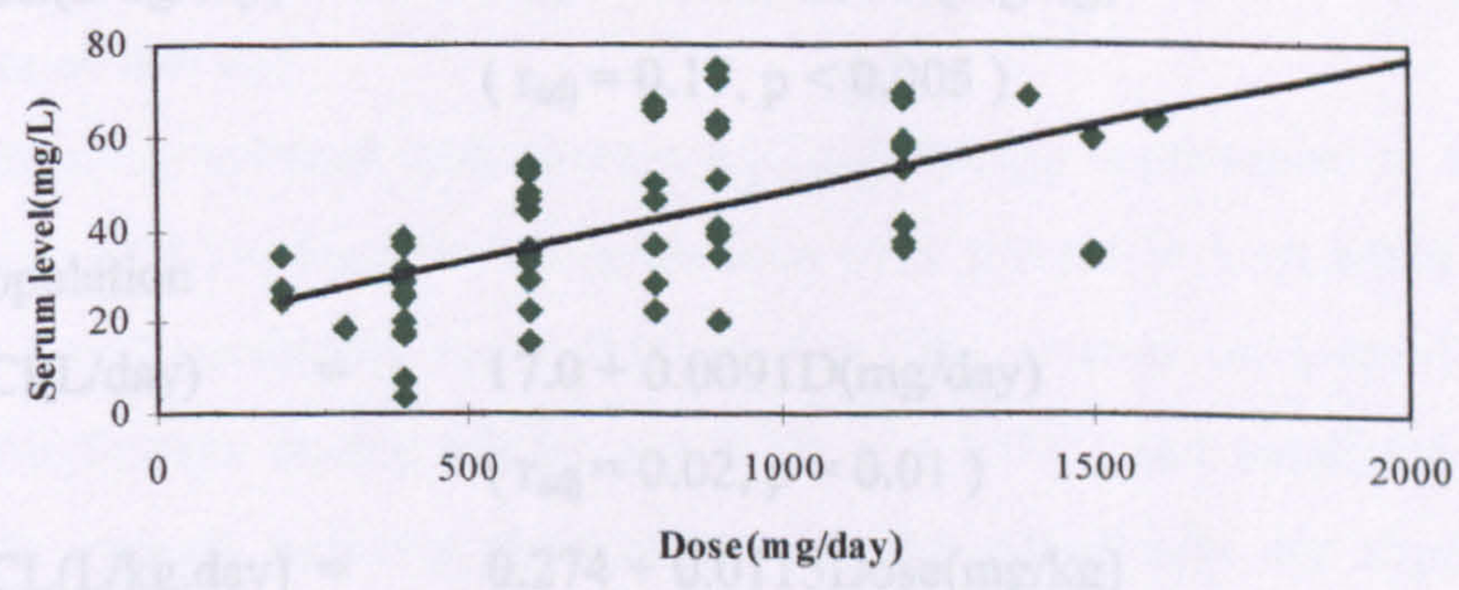


Figure 8-10: Valproic acid dose and serum level relationship in adult Indian patients



adult patients showed a considerably high degree of interindividual variation in the studied population.

Table 8-4 shows the correlation between dose in mg/day and mg/kg with serum levels in adult and paediatric patients according to ethnicity. Overall, dose and serum level relationship in all patient groups were found to be statistically insignificant. However, the only exception was observed for Indian paediatric patients which clearly showed a significant relationship(r_{adj} = 0.60, p = 0.00) between daily dose(mg/day) and serum level.

Poor correlation between dose and serum level can be associated with high interindividual variation. Interindividual variation was clearly demonstrated by the wide scatter of data points from the plots of dose and serum levels in all ethnic groups(figure 8-5 to 8-10). Evidence about the scale of interindividual variation was highlighted by the coefficient of variation(mean serum level:dose(mg/day)) ranging between 48.41% - 57.85% and 42.87% - 49.13% for paediatric and adult patients.

8.3.2 Dose and clearance relationship

The regression equations that determined the linear relationship between clearance(CL) and dose(mg/kg) or dose(mg/day) for the paediatric and adult population were as follows;

I. Paediatric population

CL(L/day) = 7.84 + 0.0055Dose(mg/day)

(r_{adj}= 0.06, p < 0.005)

CL(L/kg.day) = 0.237 + 0.0098Dose(mg/kg)

(r_{adj} = 0.17, p < 0.005)

ii. Adult population

Cl(L/day) = 17.0 + 0.0091D(mg/day)

(r_{adj} = 0.02, p = 0.01)

CL(L/kg.day) = 0.274 + 0.0115Dose(mg/kg)

(r_{adj} = 0.06, p < 0.005)

The above equations showed no significant linear relationship existed between clearance and dose in either population.

Table 8-5 shows the correlation of clearance with respect to dose according to age and ethnic groups. A non-significant linear relationship was observed all groups. These findings demonstrate that the poor correlation between valproic acid clearance and dose is not characteristic of a dose-dependent relationship.

Table 8-5: Dose and clearance(CL) relationship in adult and paediatric patients

Predictors	Malays	Chinese	Indians
I. Paediatric			
a) Regression equation (dose,mg/kg)	CL=7.10+0.0077Dose	CL=5.70+0.0068Dose	CL=10.1+ 0.0311Dose
R-square(adj)	13.3%	14.6%	0.0%
F value	17.55	11.74	0.86
p-value	0.00	0.00	0.36
b) Regression equation (dose,mg/day)	CL=0.25+ 0.0110Dose	CL=0.24 +0.0098Dose	CL=0.37+ 0.0035Dose
R-square(adj)	23.0%	16.9%	0.0%
F-value	35.28	49.67	0.86
p-value	0.00	0.00	0.36
II. Adults			
a) Regression equation (dose,mg/kg)	CL=-0.30 +0.0108Dose	CL=0.32 +.0.0095Dose	CL=0.21 +0.0132Dose
R-square(adj)	3.6%	2.8%	11.9%
F-square	4.24	3.14	10.61
p-value	0.04	0.08	0.00
b) Regression equation (dose,mg/day)	CL=18.2+ 0.0088Dose	CL=18.8+ 0.0091Dose	CL=15.3+ 0.0078Dose
R-square(adj)	1.0%	1.7%	2.3%
F-value	1.05	2.30	2.69
p-value	0.18	0.13	0.10

8.3.3 Influence of therapy

The relationship between monotherapy and polytherapy is presented in figures 8-11 , 8-12, 8-13 and 8-14. Significant correlations were present in both adult(r_{adj} = 0.58,$p < 0.005$) and paediatric($r_{adj} = 0.60$, $p < 0.005$) patients on valproic acid monotherapy. Polytherapy treated adult($r_{adj} = 0.38$, $p < 0.005$) and paediatric(r_{adj} = 0.35, $p < 0.005$) patients however showed a weak but statistically not significant relationship.

The mean serum level:dose ratios(sd) for monotherapy and polytherapy treated paediatric patients were 0.13(0.05) and 0.11(0.07) while the corresponding coefficient of variation were 50.98% and 61.99%. Values obtained for adults on monotherapy and

polytherapy were 0.07(0.03) and 0.05(0.02) with a coefficient of variation of 38.41% and 45.52%. These results showed that interindividual variations were highly distinctive in both monotherapy and polytherapy treated patients. Adults patients however were observed to display lower interindividual variations than paediatric patients.

8.3.4 Influence of ethnicity

The method described by Greenland (1987) requires essentially a linear relationship between two variables and uses the slope to estimate the trend of measured effect. The effect of ethnicity was determined by comparing the trend from the more reliable linear relationship of dose(mg/day) and serum level in monotherapy treated patients. The final result of analysis is presented in table 8-6.

Table 8-6: Summary estimate of slope of dose and serum level relationship in various ethnic groups

Age-group	**Malay**	**Chinese**	**Indian**	**Final results**
I. Paediatric				
-n	87	48	44	
-slope	0.0851	0.0679	0.0835	
-variance	0.00006	0.00008	0.00008	
-weights	16666.7	12500	12500	
-Summary slope	-	-	-	0.0795
-Q statistic				2.4046
-df				2
-p				0.15
-95% C.I				0.07-0.09
II.Adult				
-n	32	16	24	
-slope	0.0476	0.0384	0.0346	
-variance	0.00004	0.0001	0.00003	
-weights	25000	8333.3	33333.3	
-Summary slope	-	-	-	0.0399
-Q-statistic				2.4372
-df				2
-p				0.15
-95% C.I				0.03-0.05
Iii. All patients				
-n	119	64	68	
-Summary slope	-	-	-	0.0559
-Q-statistic				24.9640
-df				5
-p				<0.00
-95% C.I				0.05-0.06

Abbreviation: C.I , confidence interval

Figure 8-15: Valproic acid dose and serum level relationship in monotherapy treated Malay paediatric patients

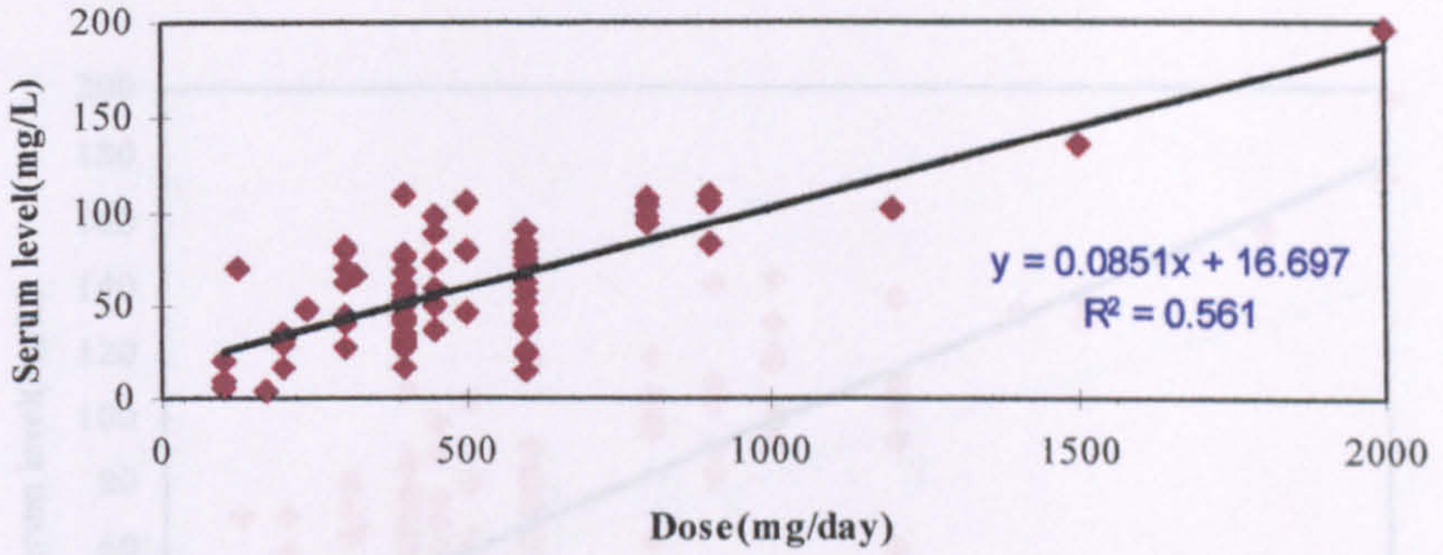


Figure 8-16: Valproic acid dose and serum level relationship in monotherapy treated Chinese paediatric patients

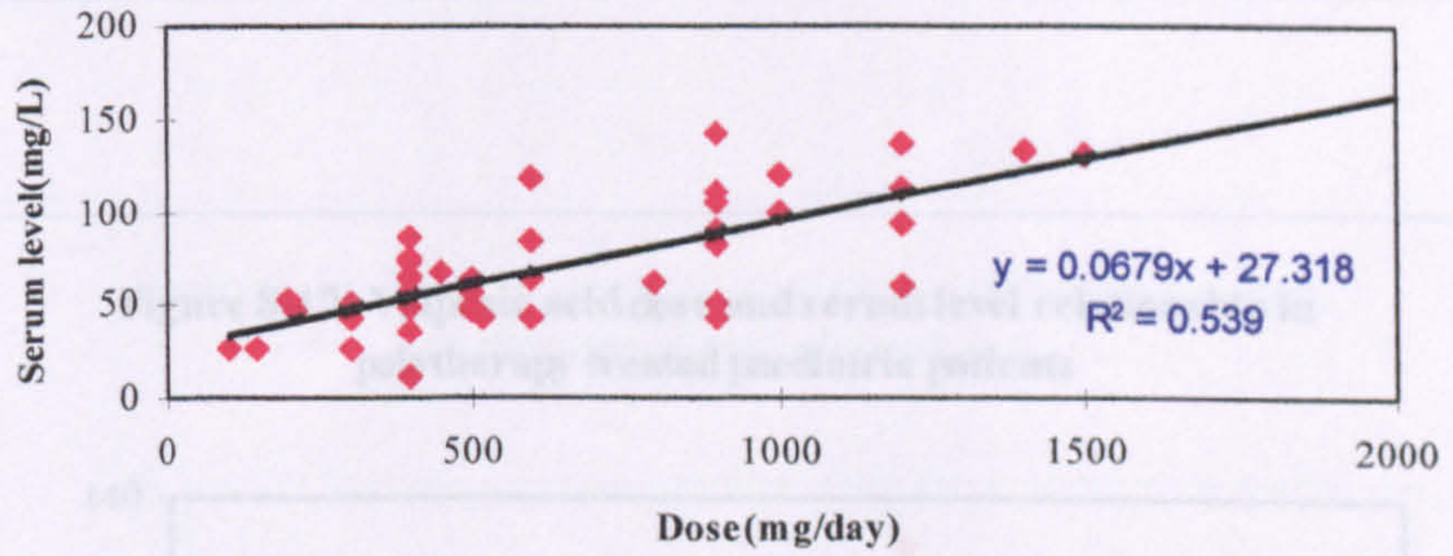


Figure 8-17: Valproic acid dose and serum level relationship in monotherapy treated Indian paediatric patients

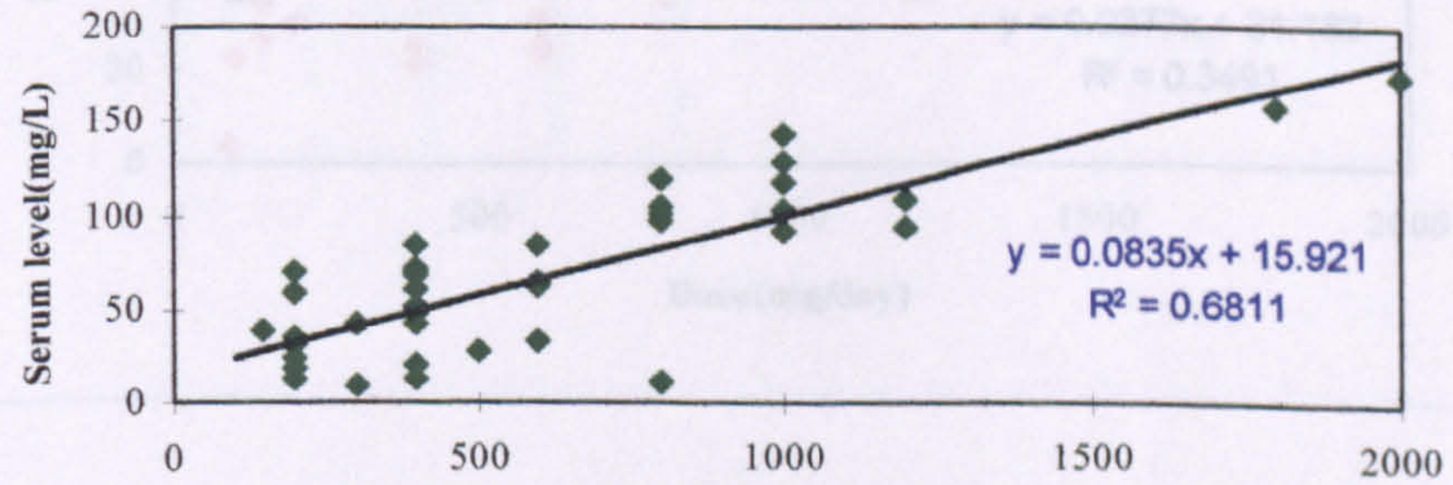


Figure 8-11: Valproic acid dose and serum level relationship in monotherapy treated paediatric patients

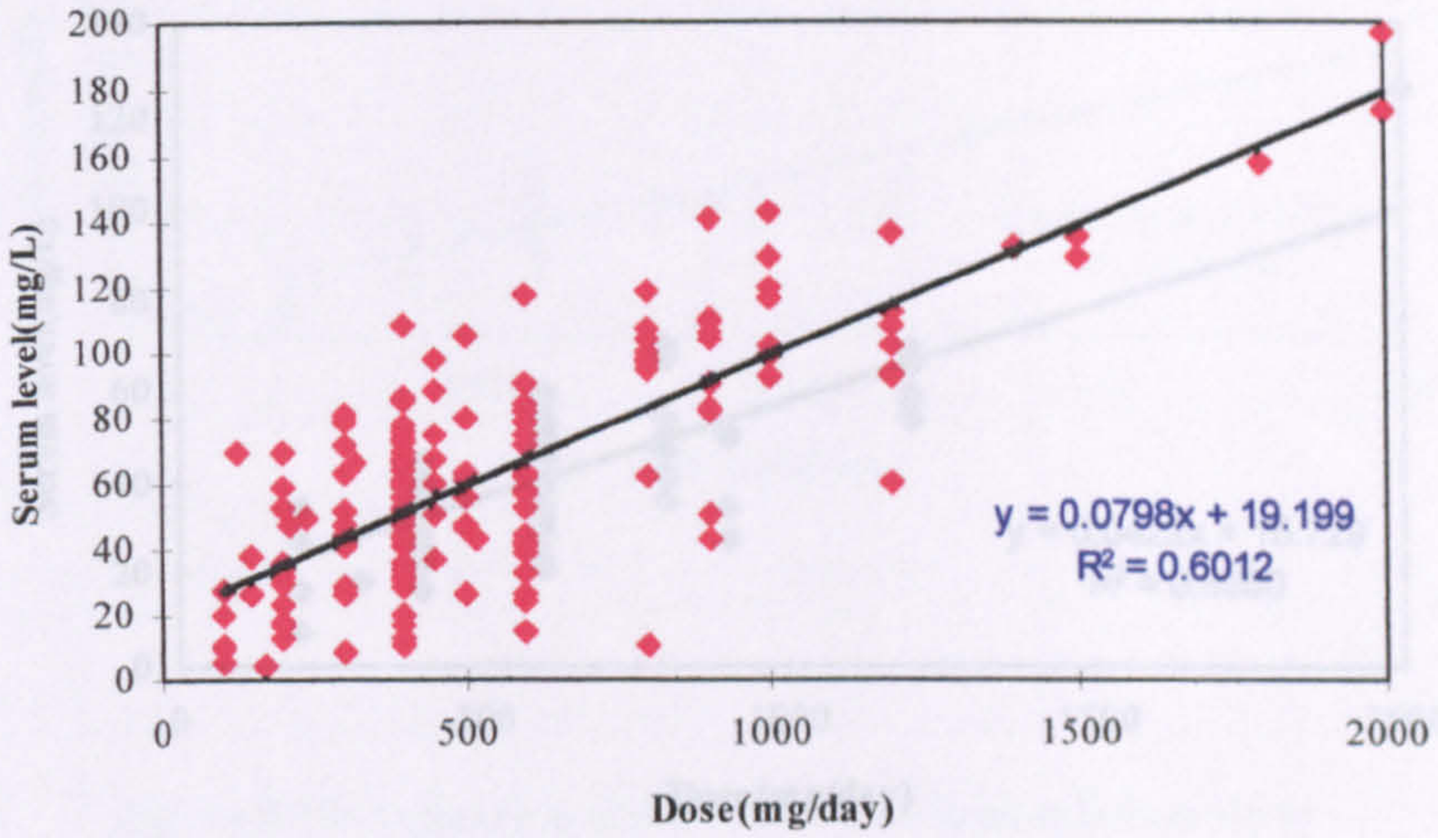


Figure 8-12: Valproic acid dose and serum level relationship in polytherapy treated paediatric patients

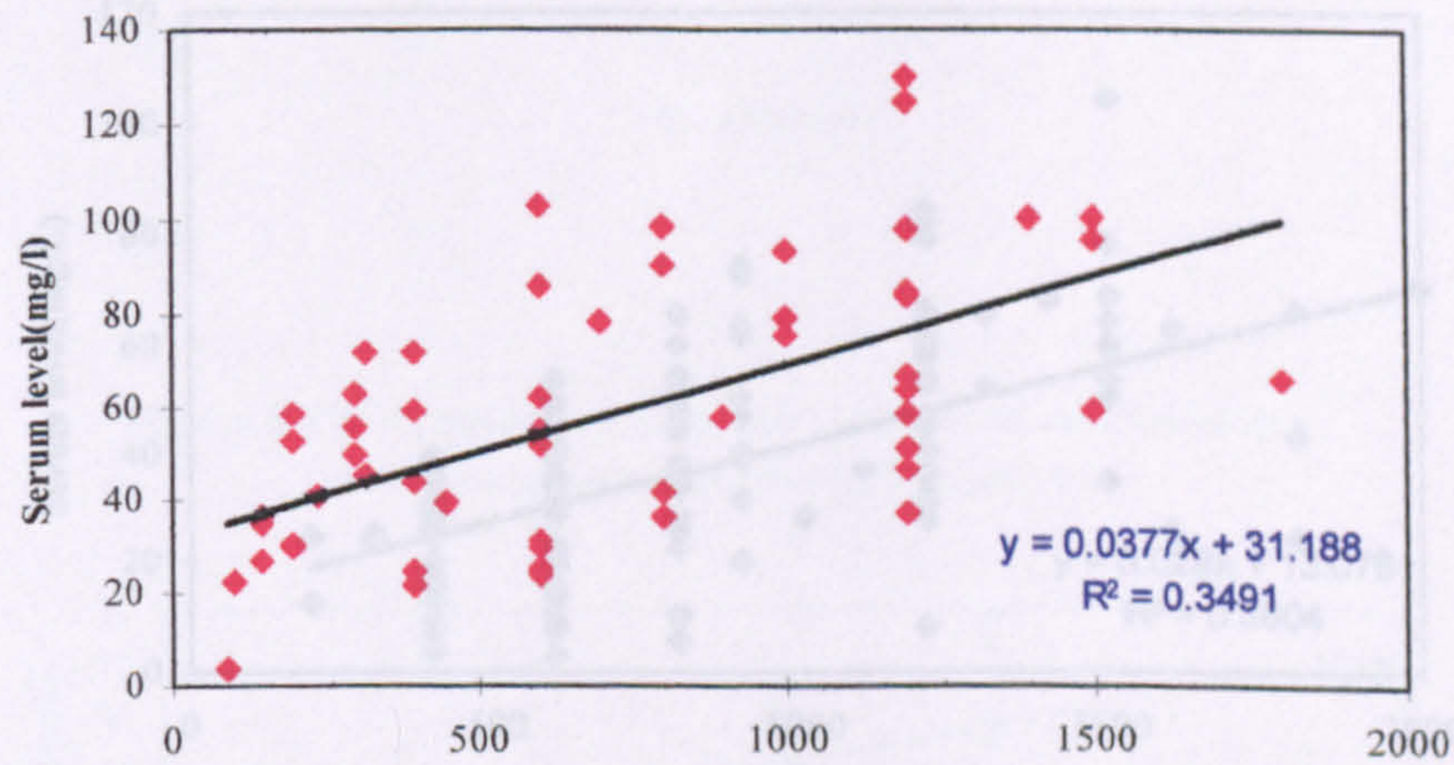


Figure 8-13: Valproic acid dose and serum level relationship in monotherapy treated adult patients

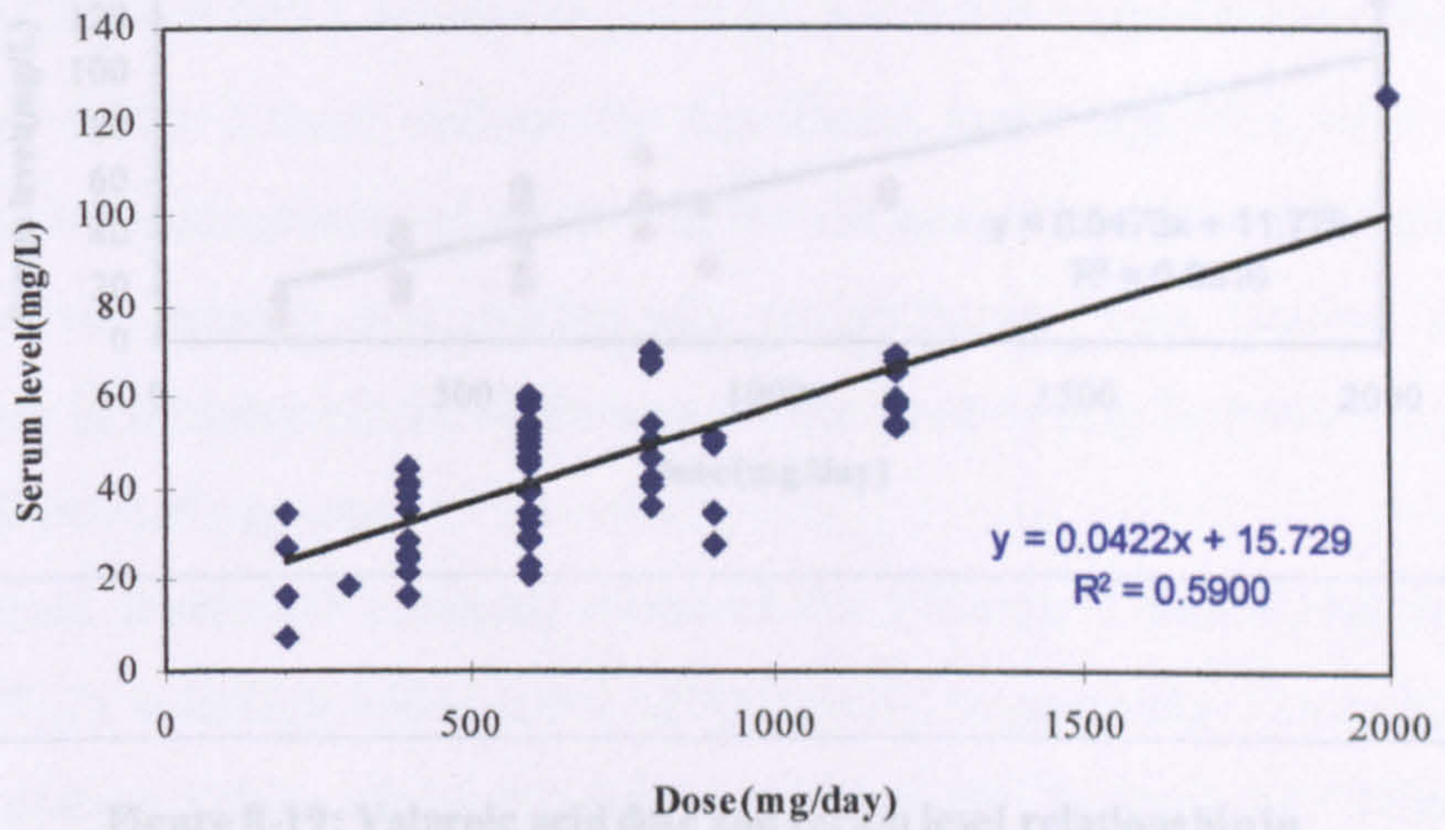


Figure 8-14: Valproic acid dose and serum level relationship in polytherapy treated adult patients

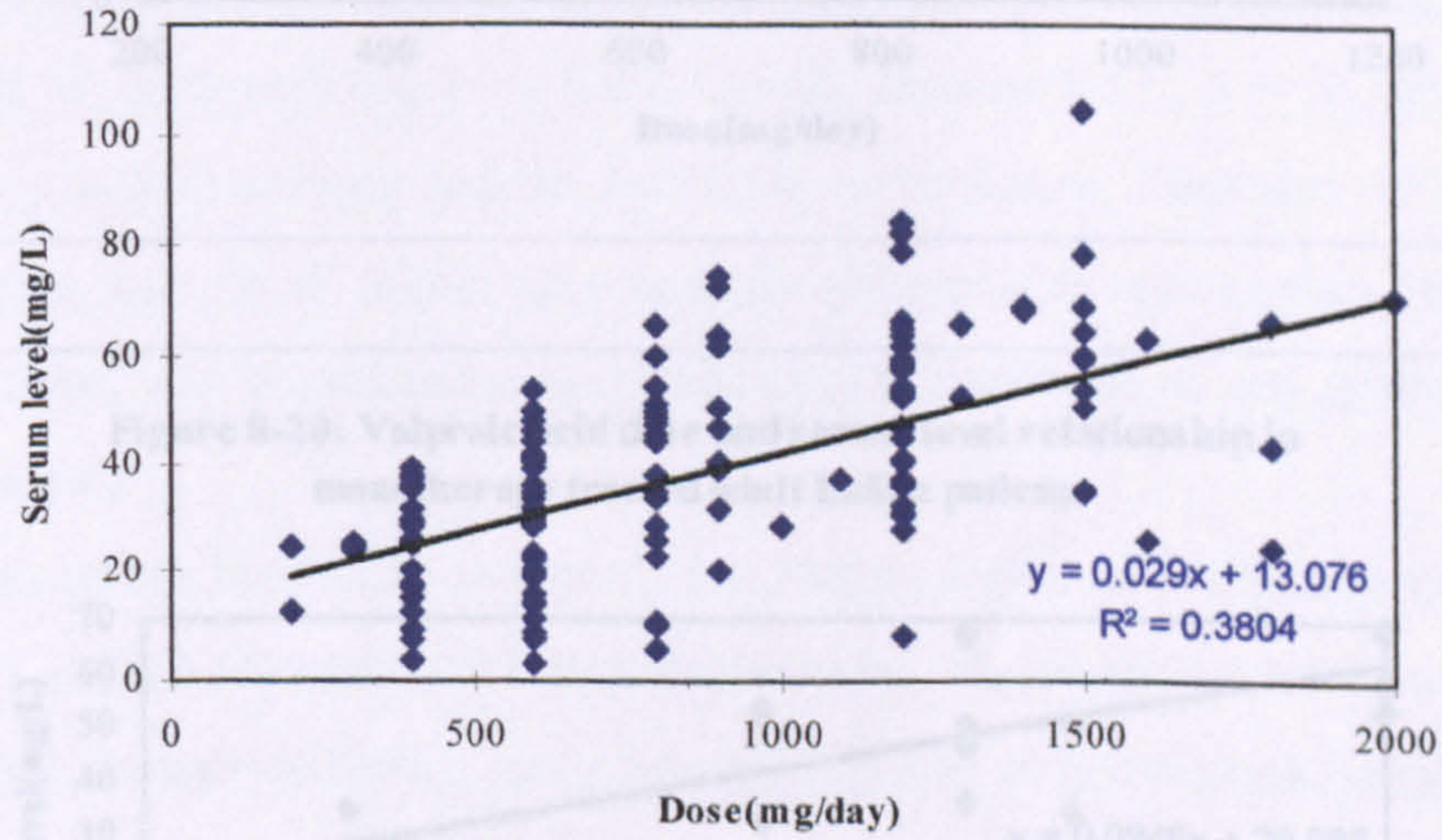


Figure 8-18: Valproic acid dose and serum level relationship in monotherapy treated adult Malay patients

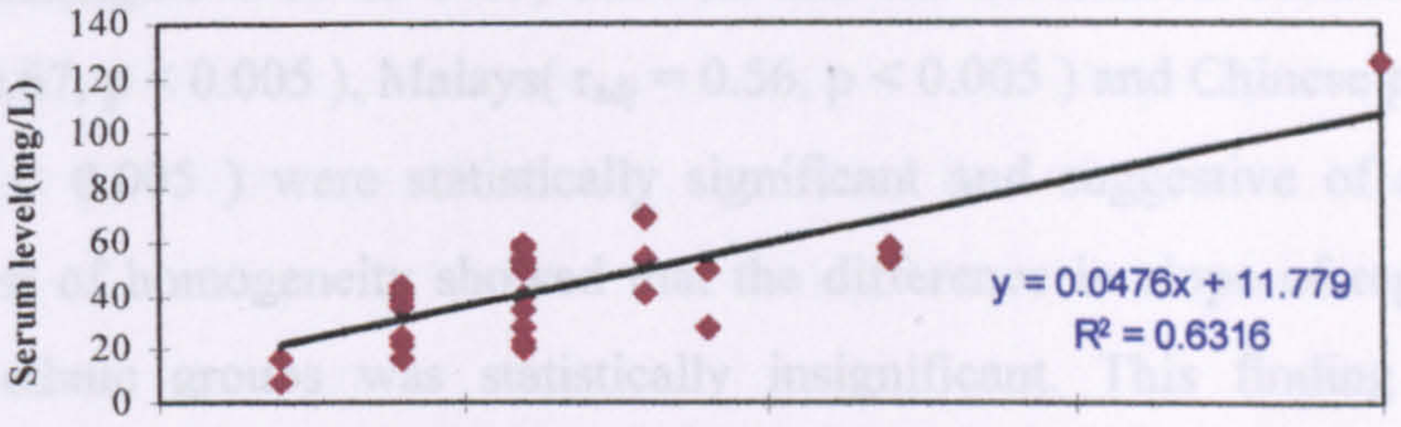


Figure 8-19: Valproic acid dose and serum level relationship in monotherapy treated adult Chinese patients

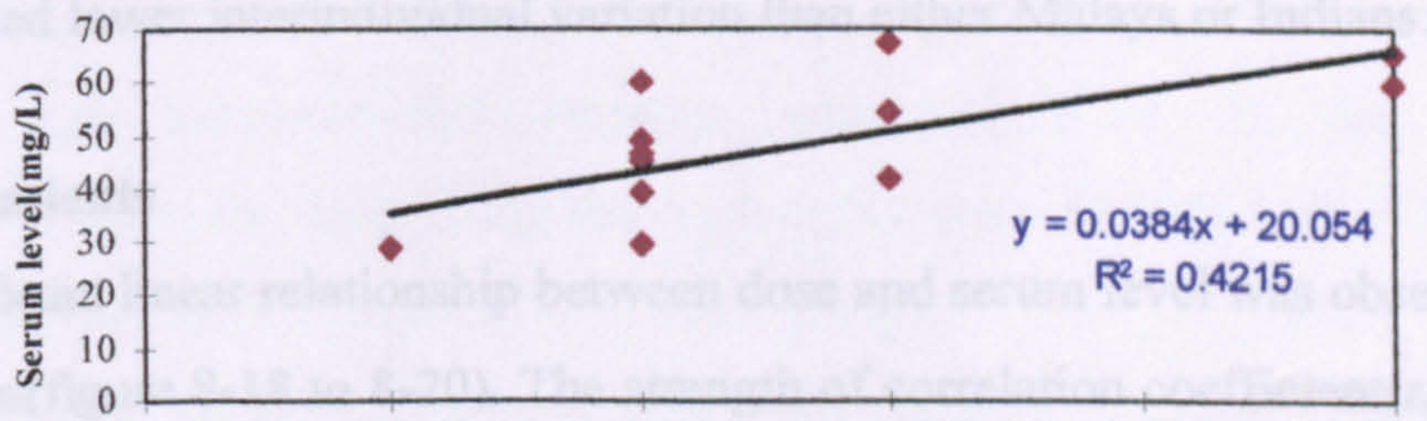
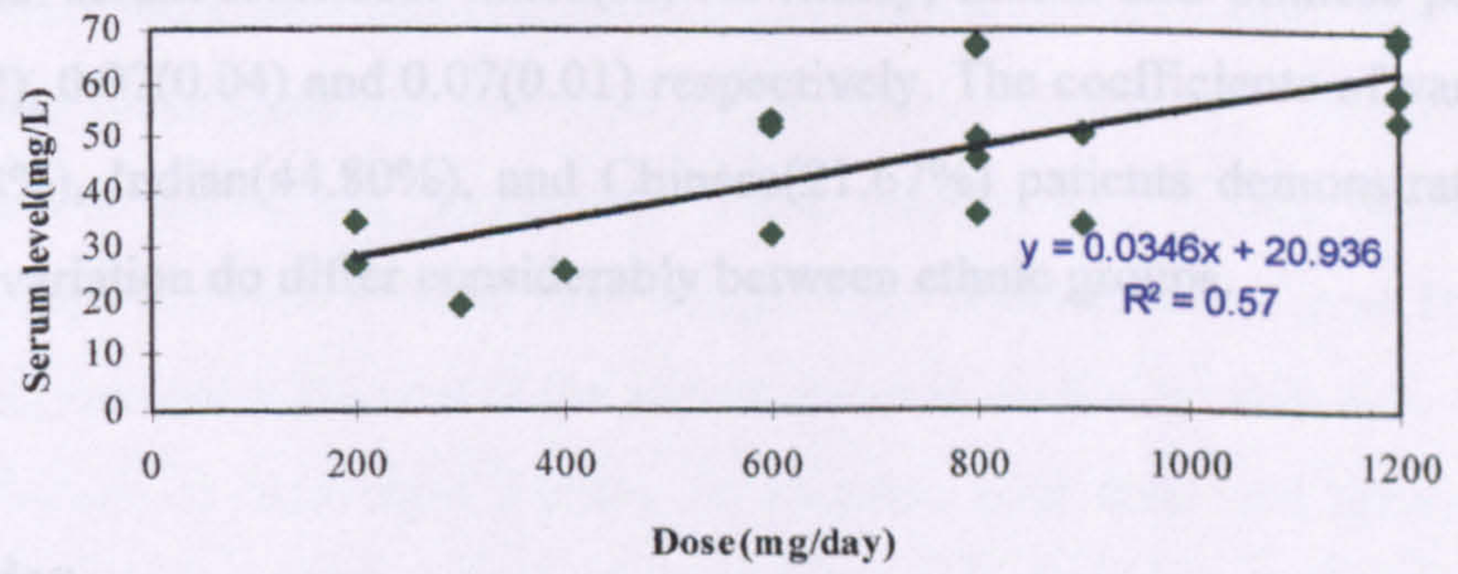


Figure 8-20: Valproic acid dose and serum level relationship in monotherapy treated adult Indian patients



I. Paediatric patients

The relationship of dose(mg/day) and serum level of monotherapy treated paediatric patients(figure 8-15 to 8-17) showed that the correlation coefficient for Indians ($r_{adj} = 0.67, p < 0.005$), Malays($r_{adj} = 0.56, p < 0.005$) and Chinese patients($r_{adj} = 0.533, p < 0.005$) were statistically significant and suggestive of a linear relationship. Test of homogeneity showed that the difference in slope of regression lines between ethnic groups was statistically insignificant. This finding clearly indicate that there is no inter-ethnic difference in the relationship between dose and serum level in all patient's groups.

Mean serum level:dose ratio(sd) obtained for Malays, Chinese and Indians were 0.1311(0.0717), 0.1283(0.0534) and 0.1230(0.0654) respectively. Corresponding coefficient of variation values of 54.69%(Malays), 41.62%(Chinese), 53.21(Indians) showed that interindividual variation was prominent in all ethnic groups. However, Chinese displayed lower interindividual variation than either Malays or Indians.

II. Adult patients

A significant linear relationship between dose and serum level was observed in all ethnic groups(figure 8-18 to 8-20). The strength of correlation coefficient(r_{adj}) was however highest in Malays(0.63, $p < 0.005$), with Indians(0.57, $p < 0.005$) and Chinese(0.42, $p < 0.005$) coming second and third respectively. Summary effect on slope of regression lines in all ethnic groups again showed the relationship between dose and serum level was homogenous and indicating the absence of any inter-ethnic differences(table 8-6).

The mean serum level:dose ratios(sd) for Malay, Indian and Chinese patients were 0.07(0.02), 0.07(0.04) and 0.07(0.01) respectively. The coefficients of variation in Malay(34.03%), Indian(44.80%), and Chinese(21.67%) patients demonstrate that interindividual variation do differ considerably between ethnic groups.

8.4 Discussion

Interindividual variation observed at similar doses has been reported by various authors [Levy RH 1984, Wulff et al 1977, Schobben et al 1975, Loiseau et al 1975, Baruzzi et al 1977]. The occurrences of high interindividual variation had led to

contradicting reports on the relationship between valproic acid dose and serum level relationship(table 8-6). Dosage selection and subsequent dosage adjustment based on this relationship is rather difficult and at most times inappropriate. Previously reported studies most often differed not only in the inclusion of different patients age groups but also as to the type of therapy. This study examined the influence of inter-ethnic differences on both of these factors in determining the probable relationship of valproic acid dose and serum level.

In the present study, the correlation between dose in either mg/day or mg/kg and serum level in mixed therapy(monotherapy and polytherapy) adult and paediatric patients of all ethnic groups showed either weak or insignificant linear relationships. These observations are in agreement with the study by McQueen et al (1982) and Haigh & Forsythe (1975). Although McQueen et al (1982) mentioned the lack of correlation was not related to irregular compliance, concomitant therapy, sampling time, dosage changes, age and weight, the probable explanation could be related to the observed high interindividual variation among patients. In this study, interindividual variation for dose in mg/day and mg/kg were 52.60% and 59.66% in paediatric patients and 52.69% to 51.87% in adult groups.

Interindividual variation has been clearly presented by Dodson et al (1981). Their results showed that although a significant relationship between dose and serum level(r value between 0.519 to 0.997) was noted for individual patients, a similar high degree of correlation was not observed when all patients' data were pooled (r = 0.42). Their report again proved that valproic acid should be individualised and that population parameters may be used as guidelines during initiating of therapy.

The present study also showed that the correlation between dose and serum level was closely dependent on the type of therapy. Patients on valproic acid monotherapy had a statistically significant linear relationship while those with concomitant therapy showed a weak and insignificant relationship. These findings were clearly in agreement with most of the earlier reported studies in table 8-7.

The influence of associated therapy on valproic acid dose and serum level relationship has been demonstrated by Sackellares et al (1981) and Henriksen & Johannessen (1982). Both of these studies showed the level of relationship of dose and serum concentration is affected by concomitant therapy in both adult and children.

Sackellares et al (1981) further compared the influence of associated therapy by observing a significant relationship(r = 0.64) in 11 patients on monotherapy and a poor insignificant correlation (r = 0.22) in 22 patients on polytherapy.

Two earlier studies by Hassan et al (1976) and Redenbaugh et al (1977) however reported a linear relationship between valproic acid dose and serum level in polytherapy treated patients. Unfortunately neither study reports the correlation coefficient of the observed linear relationship. Moreover, the plot of dose and serum levels by Rodenbaugh et al (1977) showed a wide scatter which clearly demonstrate a high degree of interindividual variation which could possibly denote a weak and statistically insignificant relationship.

Table 8-7: Studies that reported either linear or non-linear relationship between dose and serum level of valproic acid

Study	Age group	Therapy	Dose	r-value
i. No linear relationship				
Schobben et al 1975	paed + adult	polytherapy	mg/day	0.43
Johannessen SI 1977	paed + adult	polytherapy	mg/day	-
Bruni et al 1977	paed + adult	polytherapy	mg/day	0.26
Coulter et al 1980	paed	polytherapy	mg/kg	0.44
Johannessen & Henriksen 1980	paed	monotherapy	mg/kg	-
Loscher W 1981	paed + adult	mixed therapy	mg/kg	<0.5
Henriksen & Johannessen 1982	paed	monotherapy polytherapy	mg/day mg/day	- -
McQueen et al 1982	paed	mixed	mg/kg	0.1
McQueen et al 1982	paed	mixed	mg/day	0.2
Sackellares et al 1981	paed	polytherapy	mg/kg	0.2
Mesdjian et al 1984	paed + adult	polytherapy	mg/kg	-
ii. Linear relationship				
Hassan et al 1976	paed + adult	polytherapy	mg/day	-
Klotz 1977	paed + adult	monotherapy	mg/kg	0.88
Rodenburg et al 1977	paed + adult	polytherapy	mg/kg	-
Sackellares et al 1981	paed	monotherapy	mg/kg	0.64
Turnbull et al 1983	adult	monotherapy	mg/day	0.64
Mesdjian et al 1984	paed + adult	monotherapy	mg/kg	0.50
Tisdale et al 1992	adult	monotherapy	mg/kg	0.63

The relationship between dose and serum level has been reported to be dependent on time of blood sampling although trough levels(pre-dose) are commonly recommended [Chadwick 1994, Welty et al 1983]. However, several studies have shown that following the recommended time of blood sampling is not always appropriate. Baruzzi et al (1977) studied a total of 106 patients and used concentrations at both trough(pre-dose) and 5 hour post dose in determining

association. Their results showed that the correlation was poor(r = 0.10) based on trough concentrations and marginally better(r = 0.40) on concentration obtained 5 hours post dose. Bruni and colleagues similarly followed the same sampling times and found a poor correlation (r = 0.26) between dose and serum levels in 25 patients. Tisdale et al (1992) however utilised trough serum concentrations and found a significant relationship(r = 0.64). These findings clearly demonstrate the many problems in establishing valproic acid dose and serum level relationship in any population group. In this study, although serum concentration was monitored between 10.00 am to 12.00 noon for all patients irrespective of pre or post dose, a significant linear relationship was observed only on monotherapy treated patients.

Controversy over the true relationship between clearance and dose has yet to be resolved. In the present study, a poor correlation between clearance and dose was observed in all age and ethnic groups. The relationship between clearance and dose has been reported to be a non-linear exponential curve [Dodson et al 1981]. This finding has been similarly reported by Gram et al (1980) and Vadja et al (1978) and is attributed to valproic acid concentration-dependent protein binding properties. Tisdale et al (1992) however reported that the relationship is linear. They commented that their observed dose-dependent relationship is a consequence of the saturation of binding sites of the concentration-dependent protein binding properties of valproic acid.

These studies however differed in two important areas that are known to have a significant influence in the outcome of their studies, i.e, age and therapy. Dodson et al (1981) used paediatric patients on polytherapy while Tisdale et al (1992) employed adult patients on valproic acid monotherapy. These findings indicate that the relationship is probably dependent on patient's age and the type of therapy. The influence of age was moreover demonstrated by Hall et al (1981) where a similar exponential curve is reported for the association between clearance and age in paediatric patients.

The clearance of valproic acid has been reported to be affected by concomitant therapy with phenytoin, carbamazepine and phenobarbitone. The effect of phenytoin has been reported by several studies. Coulthard (1975) reported the removal of phenytoin was associated with improved in seizure control and increased serum levels.

De Wolff et al (1982) reported that the serum level/dose ratios of valproic acid were decreased by 50% in children receiving valproic acid-phenytoin combination and Henrikssen and Johannessen (1982) reported patients on this combination had an increase of 122% in serum concentration when phenytoin is withdrawn. The effect of carbamazepine and phenobarbitone withdrawal has been reported by Henriksen and Johannessen 1982 to be between 50% and 67% in serum concentration. These studies clearly demonstrate the effect of concomitant therapy on valproic acid clearance and on the relationship between dose and serum concentration.

This current study(table 8-6) showed that there was no significant inter-ethnic differences between dose and serum levels linear relationship in adult($p = 0.15$) and paediatric($p = 0.15$) monotherapy treated patients($p = 0.50$). However, high interindividual variation with a mean coefficient of variation of 50.98% and 38.42% for paediatric and adults patients indicate a high degree of diversity among individual patients in handling valproic acid therapy. Interindividual variation of adults on valproic acid monotherapy of 38.42% was also higher than the 28.86% reported by Tisdale et al (1992). This again highlight the degree of diversity of Malaysian epileptic patients thus indicating that blood monitoring is crucial during initiation of valproic therapy for epileptic patients.

The above observations clearly showed that the relationship between dose and serum level was distinctly related to interindividual variation rather than to inter-ethnic differences. However, the influence of ethnicity on interindividual variation between patients on valproic acid monotherapy in adults showed a trend where Indian patients(44.80%) having the highest coefficient of variation with Malays(34.03%) being the intermediate and Chinese(21.67%) the least. Interindividual variation for paediatric patients similarly showed that Chinese(41.62%) had the least coefficient of variation with Malay(54.69%) and Indian(53.21%) showing almost equal values. These findings showed that interindividual variation does differ between ethnic and age groups.

Evidence about the influence of age on interindividual variation is also observed in the study. Test of homogeneity between adult and paediatric patients showed that there were heterogeneity between the two groups(table 8-6). Pooled slope(mean, 95% C.I) of regression lines in paediatric(0.0795,0.0698-0.0891) and

adult(0.0399, 0.0323-0.0475) patients showed the rate of elimination is slower in younger individuals.

The effect of age in the rate of elimination of valproic acid is expected since valproic acid is primarily metabolised in the liver and hepatic function improves with maturity. Thus, higher interindividual variation observed in paediatric patients(age range 1-17 years old) is due to differing metabolic capacity between individuals.

8.5 Limitation of study

Other than problems on the design of study already discussed in chapter 5 and 6, one further limitation of this study should be mentioned. Blood samples as mentioned earlier were routinely taken between 10.00am to 12.00 noon. Since valproic acid has a short half-life of 9-15 hours [Davis et al 1994], the period of two hours may have a significant affect on the serum concentration level at the time of monitoring. The difference on the time of sampling may contribute to the variation in serum concentrations of individuals and this could affect the reliability and accuracy of the overall results although it should not have led to a systematic bias. This is especially important since establishing relationships between dose and serum levels and dose and clearance among patients groups needs both accuracy in blood sampling and drug assaying.

8.6 Conclusion

The relationship between valproic acid dose and serum level is highly variable and is dependent on patient age-group(adults and paediatric patients) and therapy. Dose given in milligram per day or milligram per kilogram body weight had no significant influence on the above relationship. The correlation between dose and serum level was however found to be highly significant in patients on valproic acid monotherapy but not for patients in polytherapy or mixed therapy(monotherapy + polytherapy).

The results of this study clearly showed that the main reason for the observed poor correlation was due to the high interindividual variation among patients. The coefficient of variation of the studied population of adult and paediatric

patients(Malay, Indian, Chinese) on valproic acid monotherapy were 54.69%, 53.21%, 41.62% and 34.03%, 44.80%, 21.67% respectively. Test of homogeneity showed that inter-ethnic differences had no significant effect on the relationship between dose and serum levels.

The relationship between clearance and dose showed no significant correlation in both monotherapy and polytherapy treated patients of all ages and ethnic groups. These results showed that the non-existence of linear relationship being not dependent on the type of therapy, age group or inter-ethnic differences. Although the true nature of its relationship is not determined, these findings disproved the claim by Tisdale et al (1992) valproic acid exhibiting dose-dependent properties although further study need to be initiated.

Finally, this study showed the clinical importance of monitoring serum concentration of valproic acid. High interindividual variation and drug-drug interaction are the two main determinants that might affect serum levels. These findings showed that individualised approach strategy is important if valproic acid is used for the treatment of epilepsy.

Chapter 9:

Phenobarbitone: Dose and serum level relationship in a multiethnic epileptic population

9.1 Introduction

Phenobarbitone was first shown to have anticonvulsant properties in 1912 by Dr Alfred Hauptmann [Hauptmann 1912]. Currently, phenobarbitone is used in partial and generalised seizures [Mattson et al 1985]. It is widely used as a sole anticonvulsant in infants and children, but in adolescent and adults, it is more commonly used as an adjunct in combination with one of the other major antiepileptic drugs.

Phenobarbitone is a classic example of a drug causing induction of hepatic microsomal enzymes [Conney 1967, Patsalos et al 1988, Wilensky et al 1982]. It increases clearances and thus decreases serum levels of other antiepileptic drugs such as carbamazepine [Welty et al 1983] and valproic acid [Bruni et al 1980, Patel et al 1980, Yukawa et al 1989]. However, drugs such valproic acid also decrease elimination of phenobarbital mainly through a direct competition for hepatic enzymes binding sites which consequently lead to elevated serum concentration [Kapetanovic et al 1981]. The drug interaction with phenytoin is complex [Patsalos & Duncan 1993].

The pharmacokinetics of phenobarbitone are well established [Evan et al 1991, Battino et al 1995]. Elimination of phenobarbitone is prolonged and the half-lives varies with age. Adults half-lives ranges from 3-5 days and in children, from 2 - 3 days. Therefore, steady state can only be accurately measured after a minimum of 20 days after adequate doses was given [Welty et al 1985, Patsalos & Duncan 1993].

The relationship between steady-state serum concentration and dose is reported to be linear in both adults [Svensmark & Buchthal 1963, Buchthal et al 1972, Strandjord & Johannessen 1977] and children [Jalling 1974, Heimann & Gladtke 1977, Painter et al 1978, Martin et al 1979]. Eadie at al (1977), however, found in a mixed age population that the relationship was curvilinear($r = 0.453$). Studies by Yukawa et al (1992) in Japanese paediatric population showed that the relationship was poor($r = 0.330$). They further accounted for their observation by high interindividual variation caused mainly by

age-dependent variations in drug disposition [Ehrnebo et al 1970] and drug-drug interactions [Patsalos & Duncan 1993].

The influence of factors such as age, sex, and concurrent therapy on dose and serum level relationship of phenobarbitone have been reported for younger patients. However, the effects of these factors were mostly investigated by examining its relationship on the ratio of serum level:dose. Rossi et al (1979) (r = 0.72) reported a strong association with age while Suganuma et al (1981) (r = 0.81) and Yukawa et al (1992) (r = 0.59) showed similar significant relationship on polytherapy treated patients. Duran et al (1988) observed that sex has no such effect.

Relationship between phenobarbitone clearance and dose have been reported as being non-linear by various authors by using semilog plots of serum concentration versus time [Viswanathan et al 1979, Butler et al 1954, Lous 1954, Svensmark & Buchthal 1963, Raven-Jonsen et al 1967]. However, a systematic study on individual epileptic patients found the relationship is non-linear in adults on monotherapy [Browne et al 1985]. Similar findings were also reported in mixed population epileptics [Duran et al 1988]. The relationship to age in children less than 15 years has been reported as being curvilinear [Yukawa et al 1992].

The above findings on the relationship between dose and serum concentration or factors that affect its relationship such as age, although important has not mentioned the effect of ethnic differences on phenobarbitone pharmacokinetics. However, phenobarbitone has been shown to display large variation(0 - 69%) in metabolism of antipyrine in identical and fraternal twins [Vessel & Page 1969]. The findings showed that the existence of a genetically controlled factor on the rate of enzyme induction properties of phenobarbitone.

The relationship between clearance and dose however suffers from lack of published reports both in children [Battino et al 1995] and adults [Browne et al 1985]. However, studies on the relationship between serum level and dose ratios (inverse to apparent clearance) showed that clearance is linear in children [Yukawa et al 1992, Suganuma et al 1981, Rossi et al 1979] and curvilinear in mixed populations [Duran et al 1988]. Both of these relationship will however be investigated in the present study on epileptic patients of Malay, Chinese and Indian origin.

This main hypothesis of this study is to prove that phenobarbitone pharmacokinetics is affected by inter-ethnic differences. Thus, this study aims to investigate the relationship of phenobarbitone dose and serum concentration in epileptic patients with different ethnic backgrounds. Determination of the relationship between clearance and dose in the three ethnic groups will be the second objective of this study. Interindividual variation and the trend of slope in the relationship between dose and serum concentration will be the main determinants to differentiate the influence of ethnicity. Finally, this study aims to propose pharmacokinetic models on which the use of phenobarbitone tailored for the multi-ethnic Malaysian population.

9.2 Methods

Patient data were obtained retrospectively using the method described in chapter 5. A total of 190 adult epileptic steady-state dose and serum data pairs were randomly selected utilising the randomisation strategy mentioned in chapter 5. Patients were divided into ethnic groups of Malay, Chinese and Indian. Their clinical characteristics are presented in table 9-1.

Non-compliant patients were determined from patients notes and plasma concentration request forms and were excluded. Details pertaining to each dose and the serum concentration selected were checked from patient notes for confirmation. The serum level was measured by the fluorescence polarization immunoassay(FPIA) method. Patients were included if they were on phenobarbitone therapy for a minimum period of 30 days.

The relationship between dose and serum level was determined by linear regression analysis and Pearsons correlation coefficient and the significance of correlation was determined using Student's *t* test [Daniel 1987]. To ease calculation, MINITAB version 10 were used for all calculation. A P value equal or less than 0.05 was considered significant. The parameter used for analysis was dose, serum level, and apparent clearance. Doses were expressed in either milligram per day(mg/day) or per kilogram(mg/kg), serum levels in milligram per liters(mg/L) and clearance in Liters per kg per day(L/kg.day). The formula to calculate apparent clearance by Wagner 1965 (as described in chapter 1, section 1.5.5) was used,

$$Clearance(CLij) = \frac{F.Dij}{Css_{ij}.\tau} \qquad \textit{(equation 7-1)}$$

where Css_{ij} is the steady state blood concentration, D_{ij} is the dose, τ is the length of the dosage interval, F is the fraction of each dose which is absorbed (bioavailability factor). Plotting Css and D_{ij} would be a straight line with the y intersect of 0 and the assumption that the other variables remains constant. F has been reported to be between 0.95 to 1 in various oral dosage forms [Nelson et al 1982, Wilensky et al 1982, Strandjord & Johannessen 1977]. For simplicity, F was taken as equal to 1. The formula assumes that at steady state (at equilibrium) the ratio of change in dose is proportionate to the ratio of change of serum concentrations. Clearance is measured in liters per kg.day (L/kg.day). Correlation of clearance and dose were evaluated by linear regression [Daniel 1987].

Table 9.1: Summary data of patients according to ethnicity

Characteristic	Malays	Indians	Chinese
No. of patients	67	38	82
Ratio of male/female	26:41	26:12	39:43
Age(yr)			
Mean,sd	38.28,11.46	39.73,14.23	37.93,11.00
Range	20.00-71.00	19.00-81.00	20.00-69.00
Weight			
Mean,sd	61.33,10.55	59.78,9.31	60.32,9.37
Range	40.00-83.00	39.00-78.00	40.00-79.00
Disease duration(yr)			
Mean,sd	15.16,11.27	14.24-10.14	14.37,9.59
Range	1.00-44.00	2.00-42.00	2.00-42.00
Epilepsy Types			
Generalised	51	32	67
Partial	13	5	14
Others	3	1	1
Therapy(M:P)	1:66	2:36	13:69
Dose(mg/day)			
Mean, sd	85.52,46.12	90.40,63.10	88.11,44.12
Range	15.00-210.00	15.00-270.00	15.00-210.00
Dose(mg/kg)			
Mean,sd	1.41,0.71	1.61,1.35	1.48,0.76
Range	0.29-3.15	0.19-6.92	0.24-4.29
Css(mg/L)			
Mean,sd	16.26,9.86	20.38,13.21	14.78,8.65
Range	0.95-48.24	3.37-45.89	1.13-46.70
CL(L/kg.day)			
Mean,sd	0.12,0.10	0.09,0.05	0.12,0.09
Range	0.04-0.63	0.02-0.24	0.04-0.67

Abbreviation: M- monotherapy, P- polytherapy

9.3 Results

Analysis of variance between ethnic group with respect to age($p = 0.74$, weight ($p = 0.70$), disease duration ($p = 0.87$), and dose in mg/kg ($p = 0.54$) showed no significant difference. Serum level ($p = 0.02$) and clearance ($p < 0.005$)

showed significant difference exist between Malays, Chinese and Indians. Mean serum level(sd) in mg/L of Malay, Chinese and Indian patients were 16.26(9.86), 14.78(8.65)mg/L and 20.38(13.21) respectively. Their mean clearances(sd) estimate in liters/kg.day were 6.99(5.65)L/kg.day, 4.79(2.97)L/kg.day and 3.42(1.50)L/kg.day.

9.3.1 Relationship between dose and serum level

Plot of dose and serum level showed that a linear relationship existed in both dose in mg/day or mg/kg(figure 9-1 to 9-2). The corresponding correlation coefficient(r_{adj}) values for dose in mg/day and mg/kg are 0.57 and 0.54. The similarities in r_{adj} values indicated the guidelines for phenobarbitone dosing in mg/kg is useful during the initial therapy and to prevent intoxication. In establishing optimum serum concentration for seizure control, dose in mg/day is sufficient.

Figures 9-1 to 9-2 are also characterised by a wide scatter of data points along the regression line which is an indicator for high interindividual variation. The mean serum level:dose ratio(sd) for dose given in mg/day and mg/kg.day for all patients was 0.20(0.10) and 11.96(5.93) and the range between 0.03-0.68 and 1.50-40.60 respectively. The calculated coefficient of variation(CV) were 50.00% and 49.58%. These high values of coefficient of variation showed a considerably high degree of interindividual variation in the studied population.

Table 9.2: Dose and serum level(SL) relationship of patients according to ethnicity

Predictors	Malays	Chinese	Indians
I. Adult			
a) Regression equation (dose,mg/kg)	SL=2.08 + 10.1Dose	SL=1.24 + 9.16 Dose	SL=8.95 + 7.11 Dose
R-square(adj)	52.6%	63.9%	51.7%
F-square	74.37	144.63	40.66
p-value	0.00	0.00	0.00
Serum level:dose ratio,sd	12.19,5.92	10.30,3.66	15.13,8.28
Range	1.58-28.22	1.50-23.00	4.96-40.60
C.V(serum level/dose)	48.56%	35.53%	54.73%
b) Regression equation (dose,mg/day)	SL=3.38 + 0.151Dose	SL=0.74 + 0.159Dose	SL=5.47 + 0.165Dose
R-square(adj)	48.8%	65.7%	61.0%
F-value	63.99	156.03	58.92
p-value	0.00	0.00	0.00
Serum level:dose ratio	0.20,0.10	0.17,0.06	0.25,0.13
Range	0.03-0.50	0.03-0.04	0.10-0.68
C.V(serum level/dose)	50.00%	35.38%	52.00%

Abbreviation: C.V, coefficient of variation.

Figure 9-1: Phenobarbitone dose and serum level relationship in adult patients

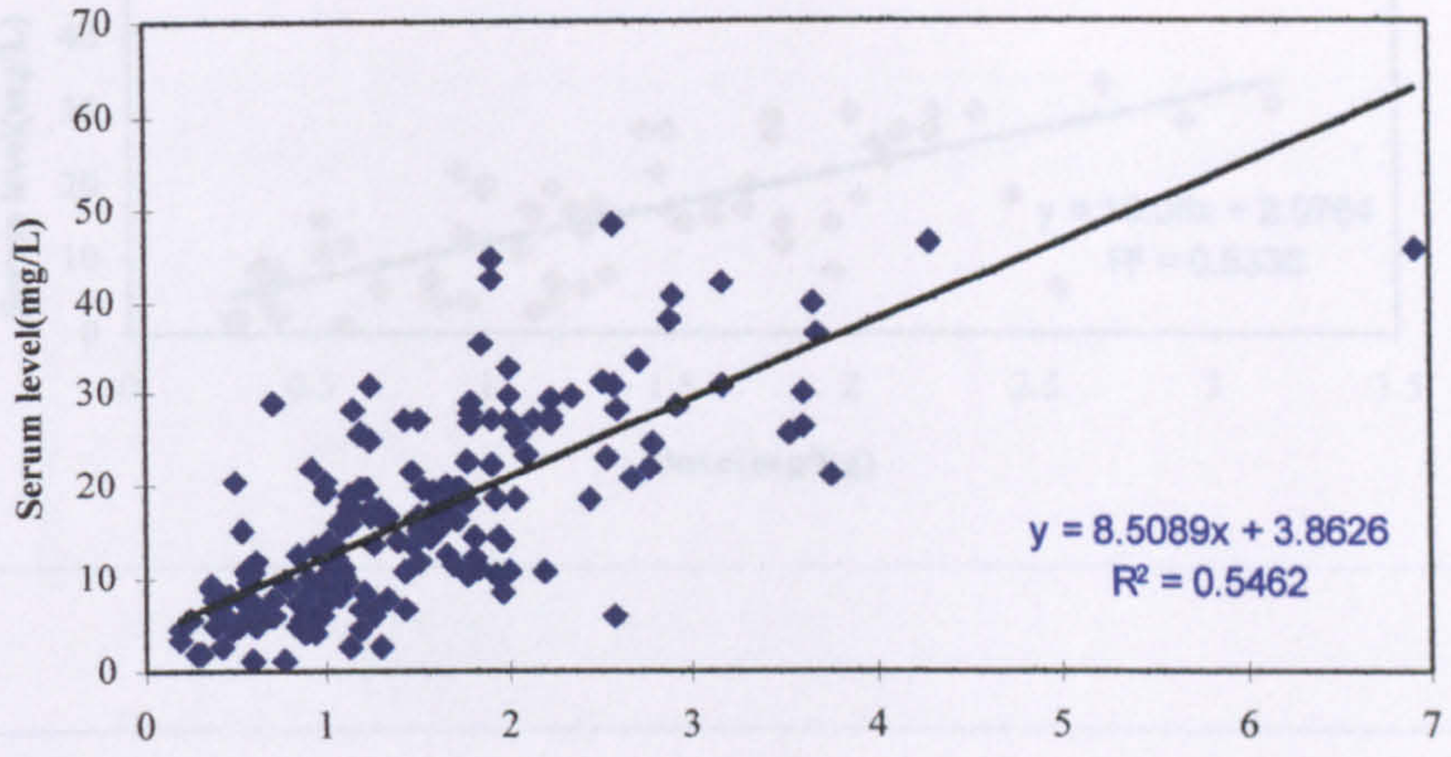


Figure 9-2: Phenobarbitone dose and serum level relationship in adult patients

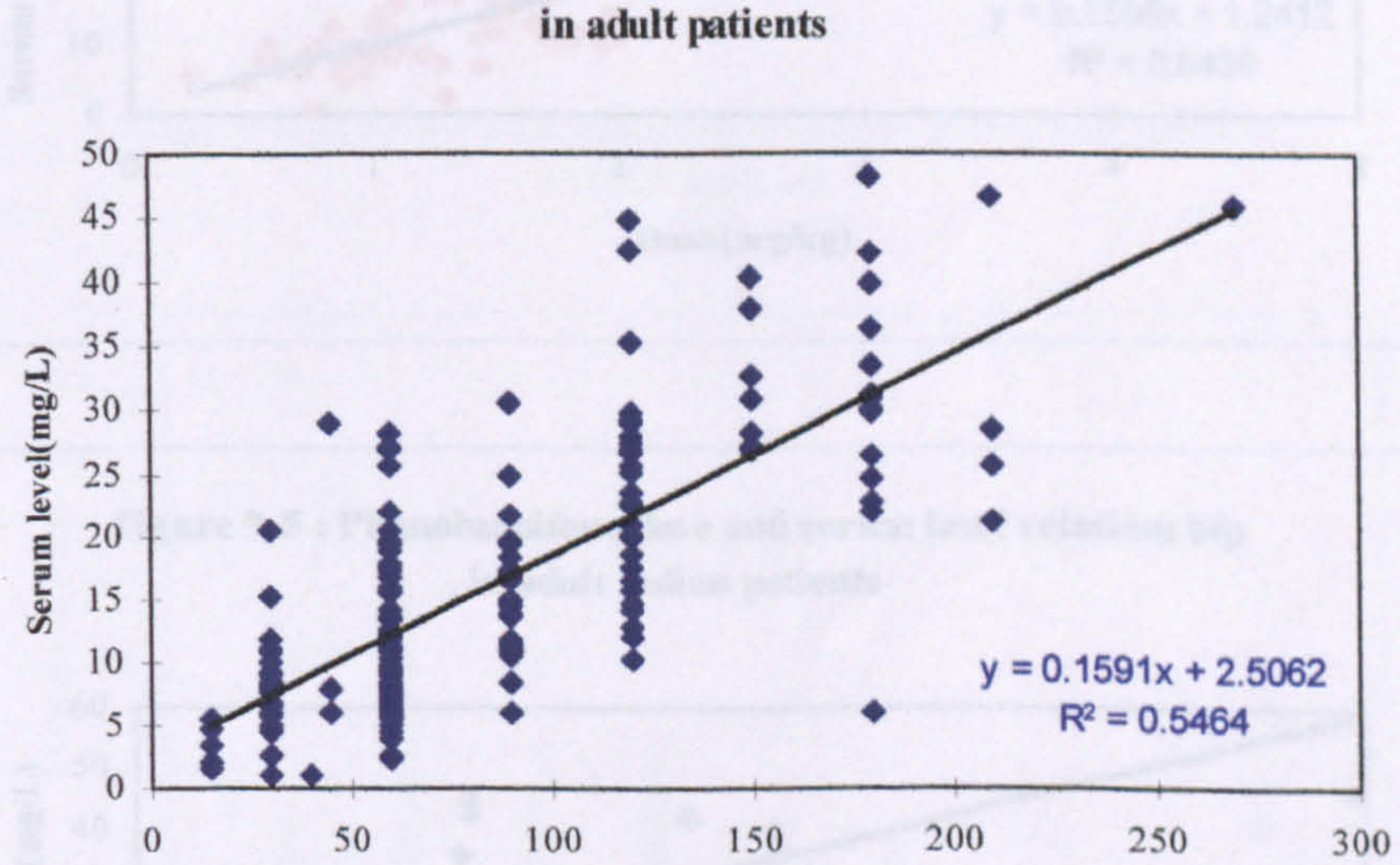


Figure 9-3: Phenobarbitone dose and serum level relationship in adult Malay patients

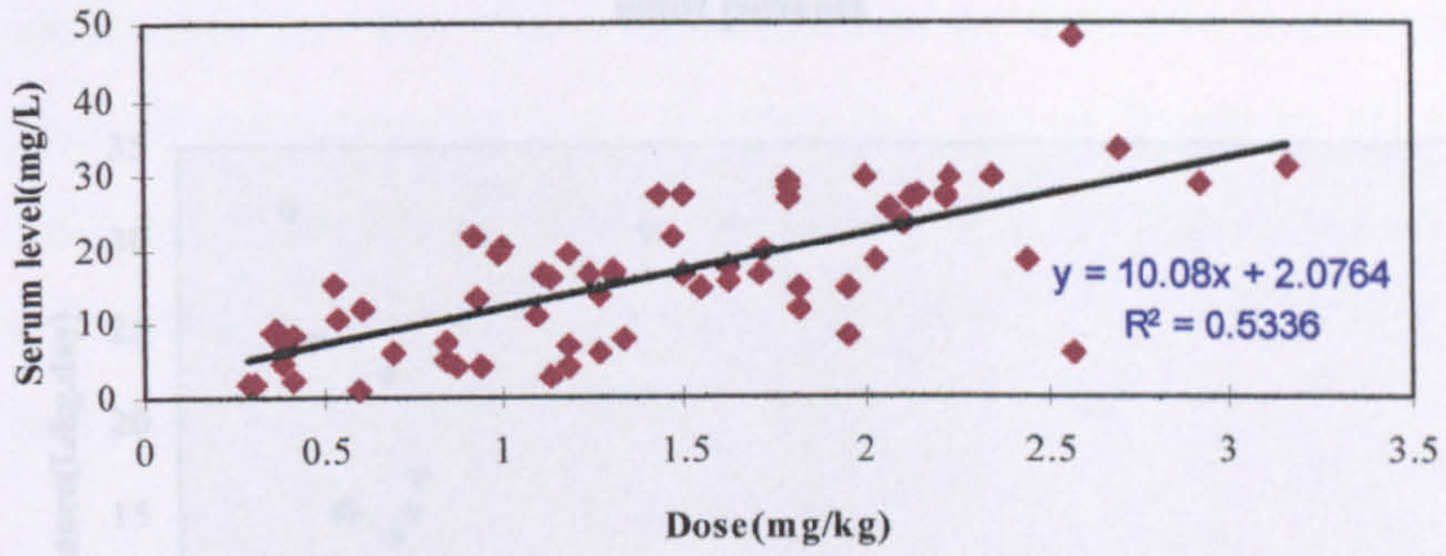


Figure 9-4: Phenobarbitone dose and serum level relationship in adult Chinese patients

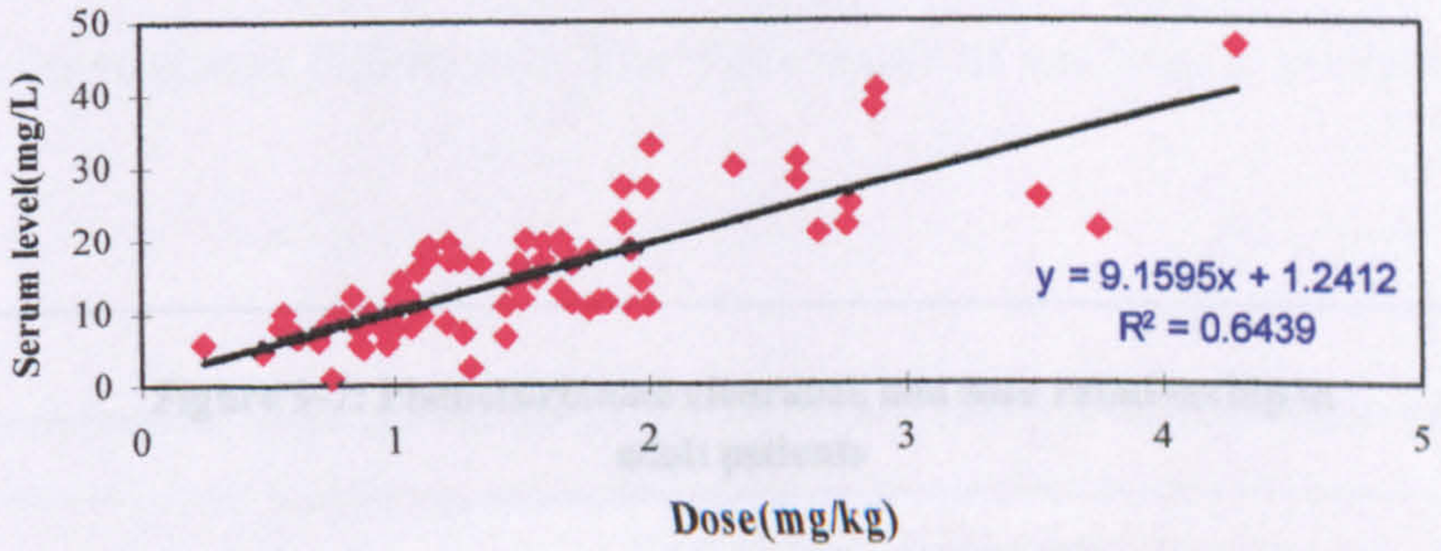


Figure 9-5 : Phenobarbitone dose and serum level relationship in adult Indian patients

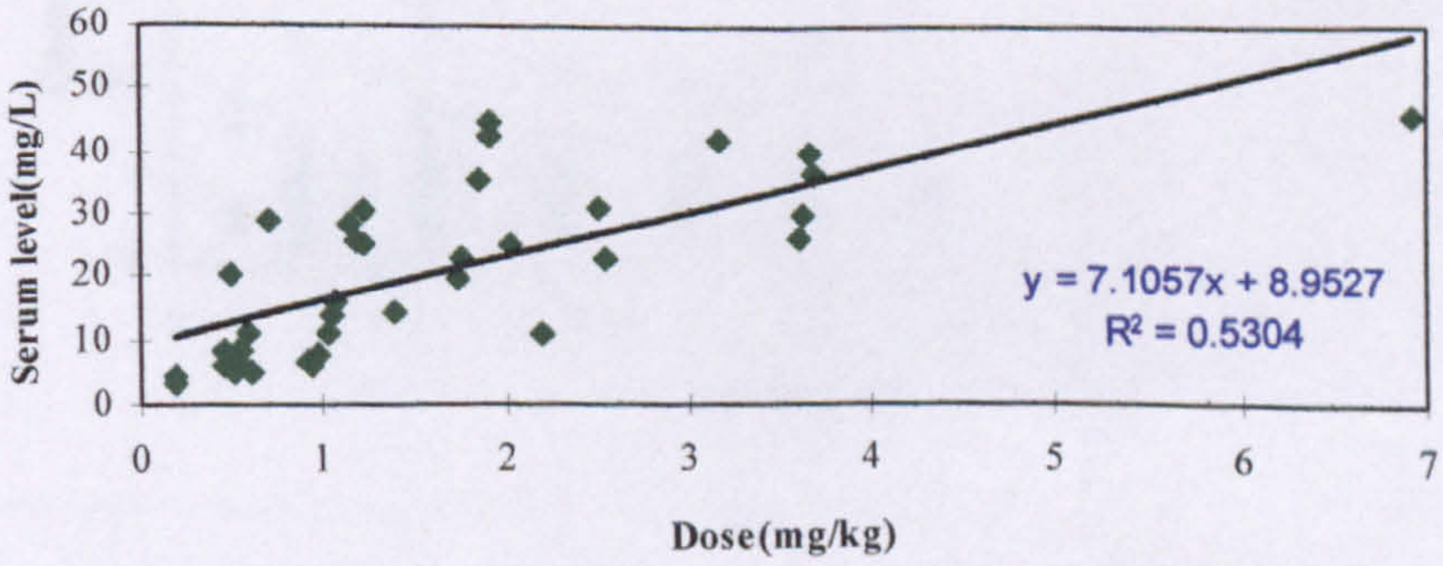


Figure 9-6: Phenobarbitone clearance and dose relationship in adult patients

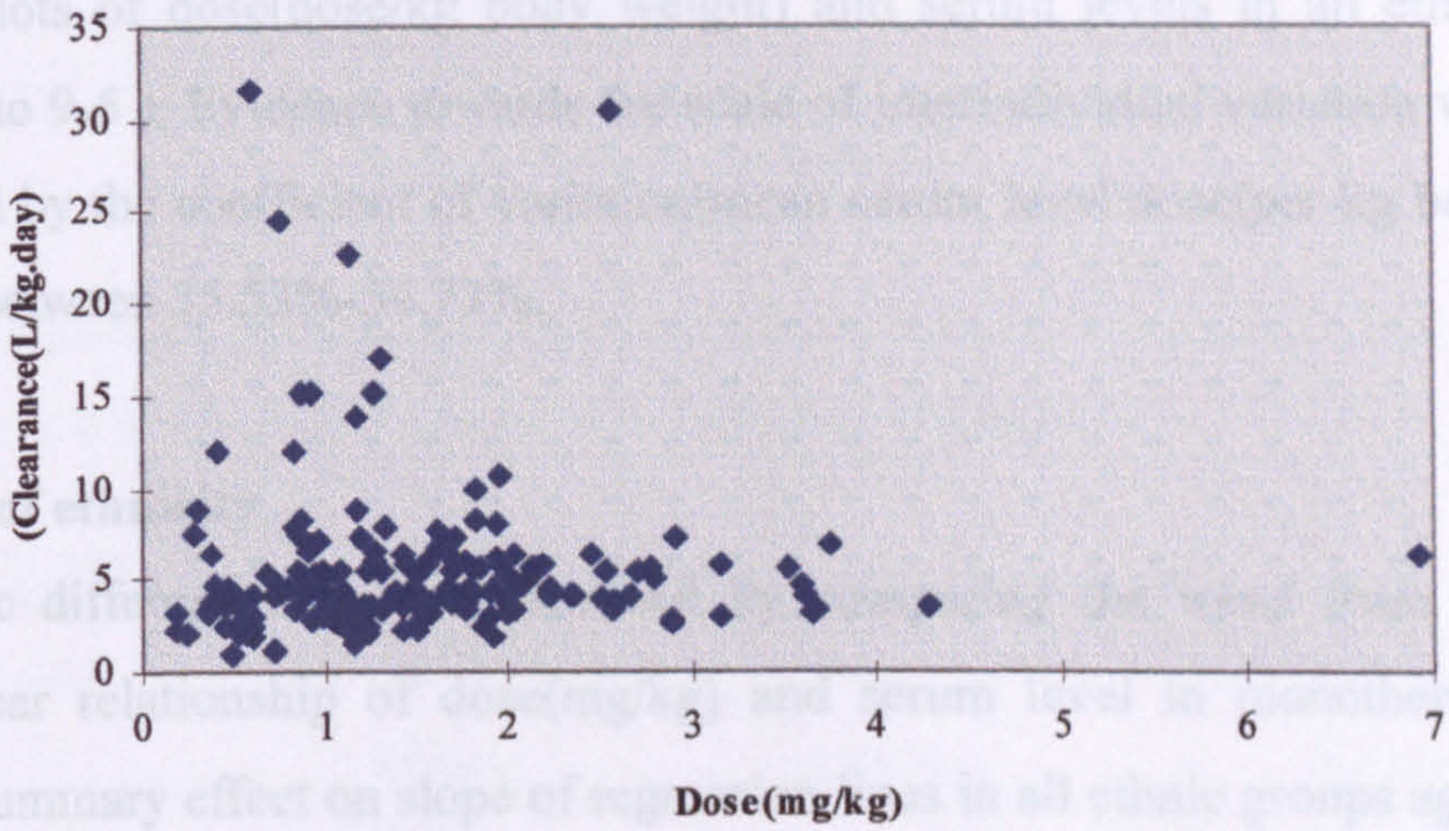


Figure 9-7: Phenobarbitone clearance and dose relationship in adult patients

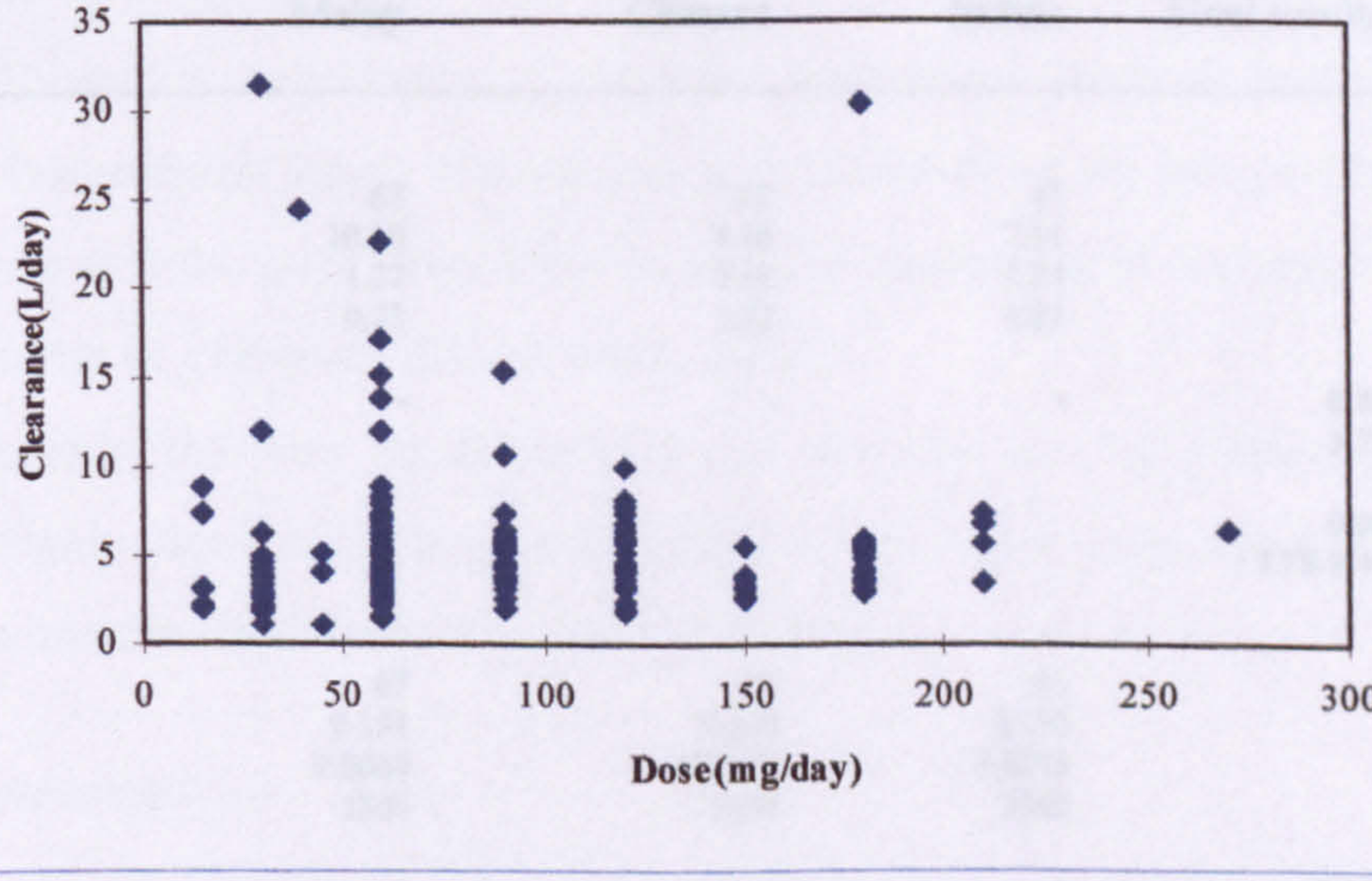


Table 9-2 shows the correlation between dose in mg/day and mg/kg with serum levels according to ethnicity. Overall, dose and serum level relationship in all patient's groups were found to be statistically significant.

Interindividual variation was clearly demonstrated by the wide scatter of data points from the plots of dose(dose/kg body weight) and serum levels in all ethnic groups(figure 9-3 to 9-5). Evidence towards the scale of interindividual variation was clearly highlighted by the coefficient of variation(mean serum level:dose(per kg body weight)) ranging between 35.53%-54.73%.

9.3.2 Influence of ethnicity

Inter-ethnic differences were determined by comparing the trend from the more reliable linear relationship of dose(mg/kg) and serum level in monotherapy treated patients. Summary effect on slope of regression lines in all ethnic groups again showed the relationship between dose and serum level was homogenous and indicated the absent of any inter-ethnic differences. The final result of analysis is presented in table 9-3.

Table 9-3: Summary estimate of slope of dose and serum level relationship in various ethnic groups

Variables	Malay	Chinese	Indian	Final results
Dose(mg/kg)				
-Sample size(n)	67	82	41	
-slope	10.10	9.16	7.11	
-variance	1.37	0.58	1.24	
-weights	0.73	1.72	0.81	
-Summary slope	-	-	-	8.86
-Q-statistic				3.75
-df				2
-p				0.08
-95% C.I				7.78-9.49
Dose(mg/day)				
-Sample size(n)	67	82	41	
-slope	0.151	0.159	0.165	
-variance	0.0004	0.0002	0.0005	
-weights	2500	5000	2000	
-Summary slope	-	-	-	0.158
-Q-statistic				0.23
-df				2
-p				0.45
-95% C.I				0.14-0.18

Abbreviation: C.I, confidence interval.

9.3.3 Dose and clearance relationship

The regression equations that determined the linear relationship between clearance(CL) and dose(mg/kg) or dose(mg/day) were as follows;

$$Cl(L/day) = 17.0 + 0.0091D(mg/day) \quad (r_{adj} = 0.02, p = 0.01)$$

$$CL(L/kg.day) = 0.274 + 0.0115Dose(mg/kg) \quad (r_{adj} = 0.06, p < 0.005)$$

The above equations showed no significant linear relationship existed between clearance and dose and is presented graphically in figures 9-6 and 9-7.

Table 9-4: Dose and clearance(CL) relationship of various ethnic groups

Predictors	Malays	Chinese	Indians
a) Regression equation (dose,mg/kg)	CL=-0.12 - 0.0047Dose	CL=5.08 -.0.0032Dose	CL=0.21 +0.0132Dose
R-square(adj)	0.0%	0.0%	11.9%
F-square	0.07	0.19	10.61
p-value	0.80	0.67	0.00
b) Regression equation (dose,mg/day)	CL=6.68- 0.0037Dose	CL=0.12- 0.0016Dose	CL=0.06+ 0.0189Dose
R-square(adj)	0.0%	0.0%	29.5%
F-value	0.06	0.02	16.34
p-value	0.81	0.90	0.00

Table 9-4 showed the correlation of clearance with respect to dose according to ethnic groups. Insignificant linear relationship was observed in all groups. These findings demonstrate that the poor correlation between phenobarbitone clearance and dose is not characterise of a dose-dependent relationship.

The relationship between clearance(CL) and dose(D) can be predicted by transforming the highly significant linear relationship of dose and serum level of the current population into the formula by Wagner et al (1965).

$$Clearance(CL) = \frac{F \times D}{Css \times \tau}$$

where F is the bioavailibility factor and equal to 1, and τ dosage regimen(per day). Since the linear relationship of the studied population is,

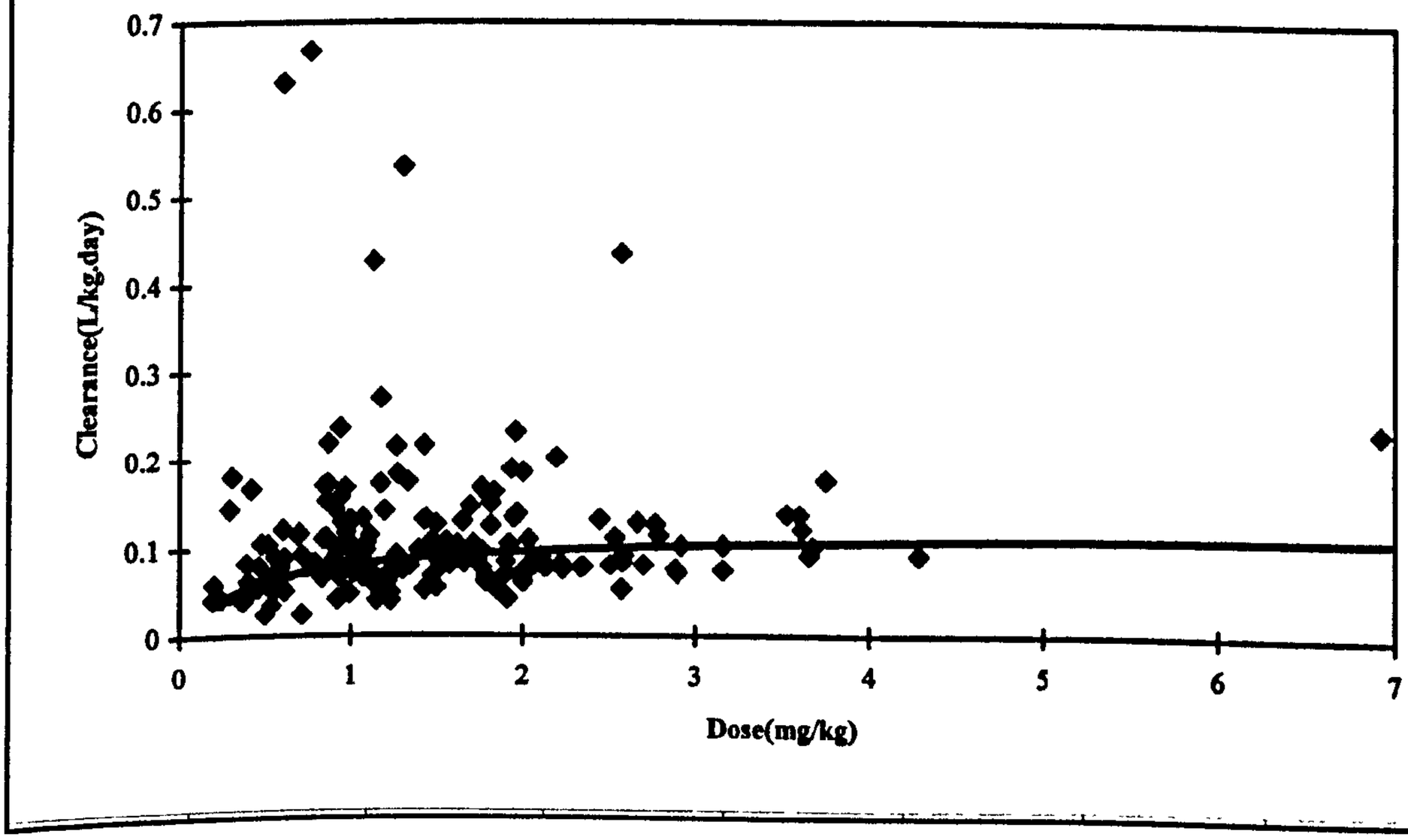
$$Css = 3.86 + 8.51 \times Dose$$

Combining the two equation, the relationship between clearance(L/kg.day) and dose(mg/kg.day) is,

$$CL(L/kg.day) = \frac{0.12 \times D}{0.45 + D}$$

The derived equation is clearly hyperbolic and characteristic of a curvilinear relationship and is depicted in figure 9-8.

Figure 9-8: Predicted clearance and dose relationship in adult epileptics



9.4 Discussion and limitation of study

The current finding of a linear relationship between dose and serum level in adult epileptics is concurrent with several earlier studies [Buchthal & Lennox-Buchthal 1972, Svensmark & Buchthal 1963, Buchthal et al 1972, Strandjord & Johannessen 1977]. The result however disagrees with that of Eadie et al (1977), Thomson and Brodie (1992) and Yukawa et al (1992) where a curvilinear or poor relationship was concluded.

Although phenobarbitone interindividual variability relationship has been attributed to age-dependent variations in drug disposition and drug interactions on younger patients [Yukawa et al 1992], the question concerning the validity of the observed serum levels in this study still needs clarification. Nevertheless, the reasons for the existence of the observed significant linear relationship can be explained by phenobarbitone established pharmacokinetics.

Phenobarbitone slow elimination which is characterised by its half-lives between 50-120 hours in adults means that its serum concentration does not fluctuate greatly throughout the dosing interval [Welty et al 1985]. The minimum time to achieve steady-state is about 20 days has made monitoring important if therapy has been initiated within the period. The period of 30 days allocated for this study is thus sufficient to attain and ensure steady-state serum concentration was achieved.

Fluctuation of phenobarbitone serum concentration can occur due to drug-drug interaction. This is especially true in this study as 91.4% of all patients were on 2 or more antiepileptic drugs. Of these 52.4% were on phenytoin, 27.8% on carbamazepine and 21.4% on valproic acid. Drug interactions involving phenytoin, valproic acid and phenobarbitone are well established although their mechanism of interaction is sometimes not defined [Patsalos & Duncan 1993]. Its interaction with carbamazepine is still unconfirmed.

A drug interaction is regarded as the modification of the effect of one drug by prior or concomitant administration of another [Patsalos & Duncan 1993]. Epileptics have been observed to receive a mean of 3.2 drugs [Guelen et al 1975] of which 84.3% were antiepileptic drugs. Since most antiepileptic drugs behave as either liver enzymes inducers(phenytoin, carbamazepine, phenobarbitone) or inhibitors(valproic acid), potentiation of any pharmacological effects due to their interactions depend if

the drug has a short or long half-lives. These interactions will reach steady-state after 4 to 6 half-lives after the interaction and monitoring before the minimum elapsed time will effectively affect serum concentration. Prescott (1980) listed the estimated minimum elapsed time for a new steady-state and complete pharmacological potentiation for phenobarbitone drug interactions with phenytoin, carbamazepine or valproic acid to be 20 days(table 9-5). This again showed the assigned 30 days in this study was adequate to achieve the desired steady-state serum concentration.

Table 9-5: Comparison of minimum elapse time required for a new steady-state and complete pharmacological potentiation to be achieved consequent to a putative enzyme inhibitory interaction

Drug	Half-lives	Period to achieve steady-state
Carbamazepine	5-15 hours	24 hours to 5 days
Phenytoin	8-60 hours	40 hours to 14 days
Valproic acid	4-20 hours	1- 5 days
Phenobarbitone	40-70 hours	10-15 days(children) 20 days(adult)

Serum concentrations of phenobarbitone have been reported to increase upon introduction of phenytoin co-medication [Lambie & Johnson 1981, Morselli et al 1971] but Eadie et al (1977) however observed no significant elevation. The study by Duncan et al (1991) showed phenobarbitone serum concentration was increased by 30%. Mechanism of interaction is probably competitive inhibition of phenobarbitone hydroxylation by phenytoin [Patsalos & Lascelles 1977, 1981].

The effect of carbamazepine on phenobarbitone is less prominent if it is given as an associated therapy. Nevertheless, Callaghan et al (1977) reported that phenobarbitone serum concentration were significantly increased if taken with both carbamazepine and phenytoin. The elevation of phenobarbitone serum level is probably to the drugs combined inductive effects on liver microsomal enzymes.

The variation in the elevation in serum level of a restrictively-cleared drug such as phenobarbitone is associated with increase of free non-protein bound component of the drug in the blood. This free unbound phenobarbitone will be easily metabolised and can then be associated with increased rate of elimination and the total serum concentration(which is commonly measured) can decrease. This decrease will nevertheless depend on the extend of drug-drug interaction [Patsalos & Duncan 1993].

These together with phenobarbitone properties of being an inducer of liver microsomal enzymes and its dose-independent elimination kinetics [Duran et al 1988], can lead to the poor correlation between clearance and dose.

The non-linearity relationship between clearance and dose in adult epileptics is in-line with that observed by Browne et al (1985). Their study however was of adult epileptics on monotherapy with a age range between 19-46 years. A similar finding is also reported by Duran et al (1988), where they utilised a mixed population of epileptics. The current finding has thus confirmed these earlier results that phenobarbitone does not exhibit a dose-dependent relationship.

The predicted curvilinear plot on the relationship between clearance and dose showed a wide variation of phenobarbitone elimination rate. These variations occur in all doses although the variation at lower doses(1-2mg/kg.day) are more prominent than doses exceeding 2mg/kg.day. The results of the present findings on a mixed age population can be explained due to differences in genetic factors [Vessel & Page (1969)] and age differences which have been attributed for the differences in elimination rate. The effect of age have been demonstrated by Boreus et al (1978) and Kutt et al (1982) where the rate of phenobarbitone in infant and children was reported to be faster in than adults.

Studies on phenobarbitone inter-ethnic differences are rare. The current results showed although there are some degrees of variation in the slope of dose and serum level, the difference is statistically insignificant($p = >0.05$). This finding showed that there is no inter-ethnic differences in epileptic patients of Malay, Chinese and Indian origin in handling phenobarbitone therapy. The result for a linear relationship in Malaysian Chinese were however similarly reported by a study on 32 Taiwanese Chinese epileptic patients of mixed age group [Lai 1985].

Nevertheless, report from a Japanese study displayed that there could still be differences in certain ethnic groups. A report of a large study by Yukawa et al (1992) of 539 Japanese paediatric patients showed a poor correlation between dose and serum level relationship and was contradictory to that published by European-based studies [Jalling 1974, Heimann & Gladtke 1977, Painter et al 1978, Martin et al 1979].

The linear relationship between serum level:dose ratios and dose in younger patients showed that Japanese [Yukawa et al 1992] presented a trend similar to

European studies [Rossi et al 1979, Eadie et al 1977]. Lee & Chan (1981) reported that in Singaporean Chinese (mostly paediatric patients), the plot of serum level:dose ratios against dose, is scattered but they did not mentioned the statistical significance of its relationship. Reports by Lai (1985) found the relationship were poorly correlated. These findings indicate a possibility that the Chinese paediatric epileptic population behave differently from similar epileptics of other ethnic groups.

The explanation for the above inter-ethnic differences can be attributed to the genetically controlled phenobarbitone liver enzymes inducing capability [Vessel & Page 1969]. Phenobarbitone inducing properties on liver microsomal enzymes has been noted to be hereditary and the differing rate of metabolism among individuals could have contributed to the above non-linear relationship.

9.5 Conclusion

The relationship between phenobarbitone dose and serum level is highly correlated in either dose given in milligram per day or milligram per kilogram body weight. This finding indicates that dosage guidelines are important during the initial introduction of therapy and further changes in dosage strategy can be based on the targeted serum concentration.

The observed non-linear correlation between clearance and dose could be due to phenobarbitone dose-independent properties. Interindividual differences and drug-drug interactions were the other reasons for the poor relationship. The relationship between clearance(Liters per kilogram per day) and dose(mg/kg body weight) were found to be curvilinear based on the formula,

$$\text{Clearance(L/kg.day)} = \frac{0.12 \times \text{Dose}}{0.45 + \text{Dose}}$$

Finally, this study showed there was no evidence of inter-ethnic differences between Malay, Chinese and Indian epileptic. The final relationship between dose and serum level for the Malaysian adult population can be predicted by the following formula;

$$\text{Serum level(SL,mg/L)} = 3.86 + 8.51 \times \text{Dose(mg/kg)}$$

The derived formula would be especially useful in predicting the targeted serum concentration for optimum therapeutic response.

Chapter 10:

Phenytoin pharmacokinetics in a multiethnic epileptic population

10.1 Introduction

Phenytoin is an anticonvulsant used for the treatment of generalised and focal seizures [Brodie 1997]. At therapeutic doses phenytoin metabolism is non-linear due to saturable or capacity-limited kinetics [Richens 1979, Bochner et al 1972, Borofsky et al 1972, Martin et al 1977, Mawer et al 1974, Richens and Dunlop 1975]. Saturable kinetics of phenytoin can be described by the Michaelis-Menten formula, which indicate that small increases in dose can cause large increases in the concentration in plasma.

The therapeutic concentration for phenytoin ranges between 10-20mg/L for adults and children [Brodie & Dichter 1997]. To achieve the targeted therapeutic range, dose administration must approach the maximum rate at which phenytoin is eliminated. The consequence of this dosing strategy is that the steady-state serum concentration shows high variability, particularly at higher doses.

Phenytoin dose-dependent pharmacokinetics depend on the estimation of the two Michaelis-Menten constants which are referred as the maximum metabolic rate(*Vmax*) and the constant equal to the plasma concentration at which the rate of metabolism is one-half the maximum(*Km*). This can only be derived at steady-state concentration(Cp_{ss}) during a dosing interval [Ludden et al 1977]. However, studies have shown that the interindividual variability of *Vmax* and *Km* is high. Reports by Allen et al (1979), Eadie et al (1976), Houghton et al (1975), Ludden et al (1977) and Martin et al (1977) showed that *Vmax* can vary between 100 to 1000mg per day and 1 to 15 mg/L for *Km*. Houghton et al (1975) even concluded that genetic differences and the effect of saturation kinetics are much more important than age, weight, height and sex.

Phenytoin metabolism had been known for many years to be genetically controlled and patients on chronic therapy developed phenytoin intoxication on the basis of a familial deficiency in hydroxylation capacity [Edeki & Brase 1995]. Arnold

and Gerber (1969) reported pronounced difference in the mean plasma half-lives between Caucasian and Negro blacks which Buchanan et al (1977) later failed to substantiate. Interethnic differences were further observed by Dam et al (1977) where the phenytoin clearance of patients from Greenland Eskimos (Danish nationality) was significantly higher than identically treated Caucasian patients from Denmark.

Ethnic differences in drug disposition of antiepileptic drugs with high interindividual variations in response such as phenytoin have been examined [Bauer & Blouin 1982, Bauer & Blouin 1983, Grasela et al 1983, Lee and Chan 1983, Andoh et al 1980, Kromann et al 1981]. Sheiner et al (1981) moreover reviewed phenytoin population pharmacokinetics and found considerable and significant differences between Japanese and European population.

Objectively, this study aims to investigate the influence of ethnicity on the pharmacokinetics of phenytoin. This study also aims to propose ethnic specific population pharmacokinetic parameters of phenytoin (*Km* and *Vmax*) for the multi-ethnic Malaysian population.

10.2 Methods

A total of 432 epileptic patients comprising of 383 adults and 49 children were included in the study. A total of 864 steady-state dose and serum data pairs (two pairs per patient) were selected utilising the randomisation strategy described in appendix 5-11. Details concerning the methods for data retrieval are described in chapter 5.

Patients were divided into groups according to age and ethnicity. Age groups were identified as children or adult. Children are defined as patients whose age is less than or equal to 18 years. Patients whose age were more than 18 years were all categorised as adults. The ethnic groups studied were Malay, Chinese and Indian and the clinical characteristics of the patients pertinent to the study are presented in table 10-1.

Non-compliant patients were excluded. The method of recognising compliance was by reviewing through reading patients notes and comments made from the plasma concentration request forms. Details pertaining to each dose and serum concentration selected were checked from patient notes for confirmation. The serum level was measured by the fluorescence polarization immunoassay(FPIA) method. Patients were also included only if they were on phenytoin therapy for a minimum period of 30 days.

The three main variables investigated were dose, serum concentration and clearance. Dose(*R*) can either be in milligrams per day(mg/day) or milligrams per kilogram body weight(mg/kg.day). Serum levels were in milligram per liter(mg/L) while clearance was described in liters per day(L/day). Data were analysed using the software available in the Minitab Statistical package, version 10.2. Differences in age, sex, disease duration and epilepsy types in each group were evaluated by one way analysis of variance(ANOVA) with the degree of significance of p <= 0.05. Coefficient of variation(CV) for interindividual variation was calculated using the following formula: CV(%) = (SD ÷ Mean) x 100, where 'SD' denotes standard deviation.

Linear regression was used to determine the influence of continuous variables such as age and weight on the Michaelis-Menten parameters(*Km* and *Vmax*). The graphic method was used to estimate *Km* and *Vmax* of each individual(figure 10-1).

Table 10-1: Summary data of patients of various ethnic groups

Characteristic	Malay	Chinese	Indian
No. of patients	171	158	104
Ratio of male/female	99:72	87:71	53:51
Age(yr)			
Mean,sd	33.96,12.56	33.21,13.38	30.81,11.27
Range	5.00-73.00	2.50-69.00	10.00-53.00
Weight			
Mean,sd	58.34,12.71	56.97,13.95	54.45,12.61
Range	11.75-90.00	10.00-87.00	10.00-87.00
Disease duration(yr)			
Mean,sd	10.84,8.71	13.86,11.50	14.71,10.13
Range	0.00-38.00	0.50-55.00	1.50-41.00
Epilepsy Types			
Generalised	127	115	85
Partial	25	28	10
Others	19	15	9
Therapy(M:P)	102:69	90:68	61:43
Dose(mg/day)			
Mean, sd	272.60,83.23	265.66,80.08	274.86,70.57
Range	45-600	15-460	100-450
Dose(mg/kg)			
Mean,sd	4.84,1.65	4.87,1.89	5.34,1.96
Range	1.39-12.50	1.25-15.00	1.55-14.28
Css(mg/L)			
Mean,sd	14.18,8.34	14.67,9.29	15.81,10.06
Range	0.90-41.29	1.09-43.99	0.89-47.20
CL(L/kg.day)			
Mean,sd	25.68,15.96	24.90,14.81	24.85,17.36
Range	3.44-111.11	5.91-93.46	6.62-148.15

Abbreviation: M- monotherapy, P- polytherapy

In order to distinguish differences between *Km* and *Vmax* values between adult and children, the method proposed by Cochran 1954 was employed. This method

basically uses the Q statistic to test for homogeneity for the summary measure of effect size and is described in Chapter 3.

The problem of sensitivity in the method of randomly selecting two dose/serum level pairs may produce unrealistic individualised values of *Km* and *Vmax*. This is clearly demonstrated by the estimated *Km* values and their distribution in Appendix 10-1 to 10-3. To address the problem, the upper and lower 10% of *Km* and *Vmax* values in each ethnic group were trimmed and the final values taken for analysis were those in the middle bracket of 80%($100 \pm 10\,\%$).

10.2.1 Data analysis

Using the Michaelis-Menten equation, for a series of dosage rate-steady-state level pairs from each individuals, the following relationship is employed.

$$R_{ij} = \frac{Vm_j \,.\, Cpss_{ij}}{Km_j + Cpss_{ij}}$$

R_{ij} is the dose rate(mg/day) for the ith pair in the jth patient, Vm_j is the maximum elimination rate of the *jth* patient, $Cpss_{ij}$ is the steady-state plasma concentration(ug/ml) measured in the *jth* patient while receiving the *ith* dosage and Km_j is the Michaelis-Menten constant for phenytoin metabolism. The parameters Vm_j and Km_j are assumed not to vary within patient but may vary between patients. *Vm* and *Km* are calculated by assuming at steady-state condition and following Michaelis-Menten relationship of;

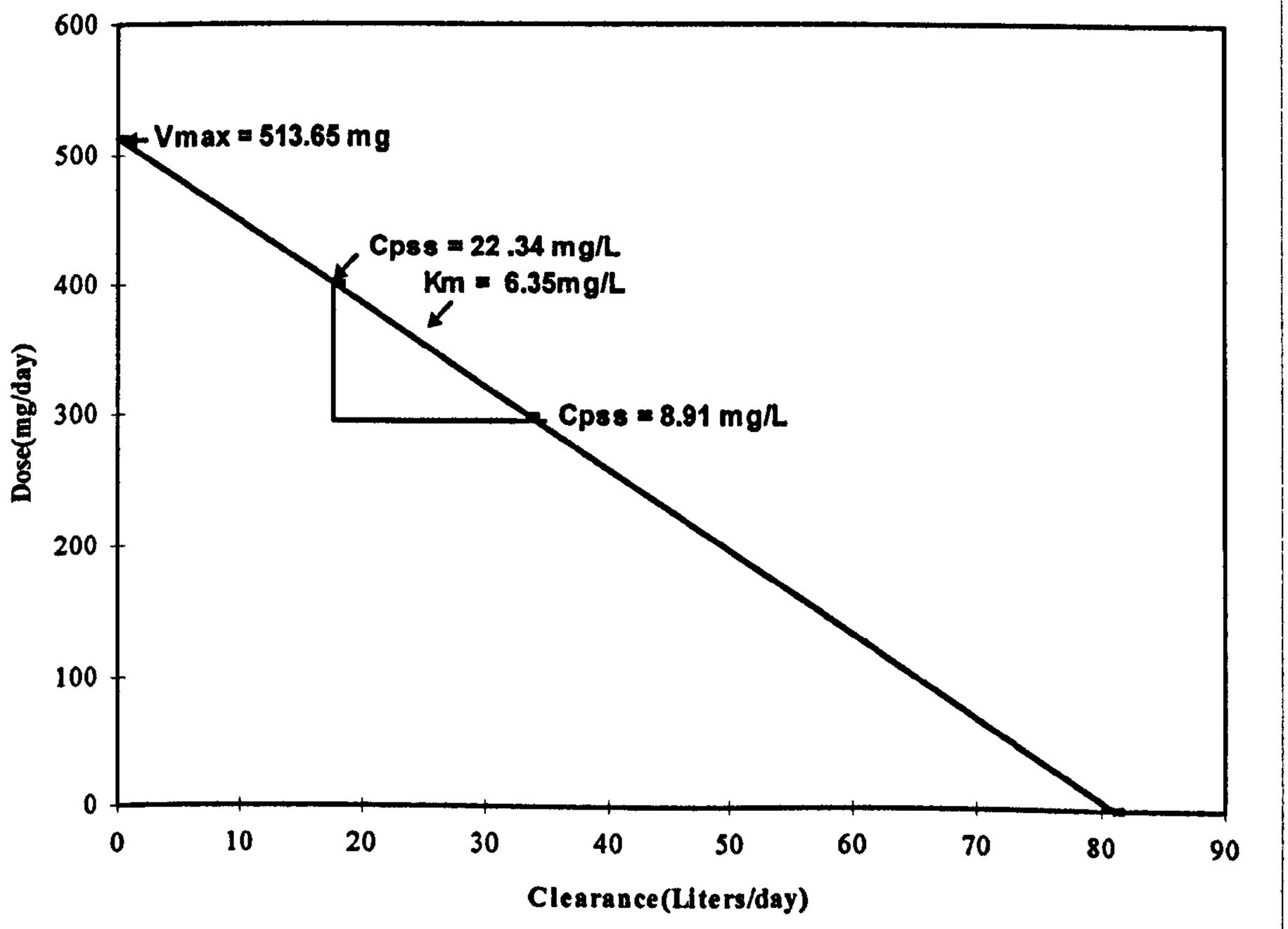
$$S\,.\,F.\,R = \frac{Vmax \;-\; Km\,.\,[\,S.\,F.\,R.]}{Css}$$

where *S.F.R* is the dosing rate, which is dependent on the salt factor(*S*) form and is equal to 1, *F* is the bioavailibility while *R* is the rate of administration(dose/day,equal = 1). For this study, bioavailibility was assumed to similar to all patients for all patients. *Vmax* is defined as the maximum rate of metabolism (metabolic capacity) and *Km* is a constant with a value equal to the plasma concentration at which the rate of metabolism is one-half the maximum.

The point of intercept on the y-axis is the value for *Vmax* (mg/day) while the slope is the value for *Km*(mg/l). Thus, at least two dose-level pairs are needed in

plotting the desired graph. The final regression equation $y = a + bx$, will represent the dose/serum level pairs, where a(constant) is the intercept to the y-axis and equal to *Vmax* and *b* is the regression coefficient or gradient signify *Km*. The graphical estimation used is presented in figure 10-1. Each individual *Km* and *Vmax* was estimated by plotting their two dose/serum concentration pairs where the gradient corresponds to *Km* and the y intersect is the *Vmax*.

Figure 10-1: Graphic estimation of individual Vmax and Km values in a female Chinese, age 17 years old who had phenytoin concentration of 8.91 mg/L and 22.34mg/L while receiving a dose of 300 and 400 mg daily.



Km and *Vmax* can also be estimated using simultaneous equations by substituting the value of *y* to serum concentration (mg/L) and *x* as the dose (mg/day) in the linear equation $y = a + bx$.

The mean estimates of *Km* and *Vmax* of each ethnic groups were then used to calculate the total daily dose needed to produce a given steady state phenytoin concentration, according to the following equation;

$$Dose = \frac{Vmax \;.\; Cpss}{Km + Cpss}$$

where dose is in mg/day, *Vmax* (mg/day), *Km*(mg/L) and *Cpss*(steady-state) serum concentration in mg/L.

10.3 Results

A total of 306 adults and 40 paediatric patients were finally enrolled for the study. The characteristics of each ethnic groups according to age are presented in table 10-2 and 10-3. Proportion of Malay, Chinese, and Indian in the adult groups were 41.18%, 35.29% and 23.53%. The percentage for paediatric patients were 27.50%, 45.00% and 27.50% respectively.

The plot of phenytoin serum concentration versus dose(mg/kg.day) is given in figure 10-2. These highly scattered graphs as expected showed that there were no obvious linear relationship between dose and serum concentration. Plots of dose(mg/day) and clearance of each patient groups also showed a wide scatter(figure 10-3). This clearly indicate that the determination of *Km* and *Vmax* should be individualised.

Analysis of variance showed that inter-ethnic differences in adults for age, weight, dose(mg/day and per/kg body weight), serum levels and clearance were insignificant($p > 0.05$). Differences in length of disease years(epilepsy years) was statistically significant($p<0.00$). Mean for length of disease years (95%, C.I) in years for Malay, Chinese and Indian adult patients were 11.21 years(10.09 - 12.33), 15.08 years(13.49 - 16.67) and 15.65 years(13.89 -17.41).

Figure 10- 2: Plot of dose and serum concentration in Malay, Chinese and Indian patients

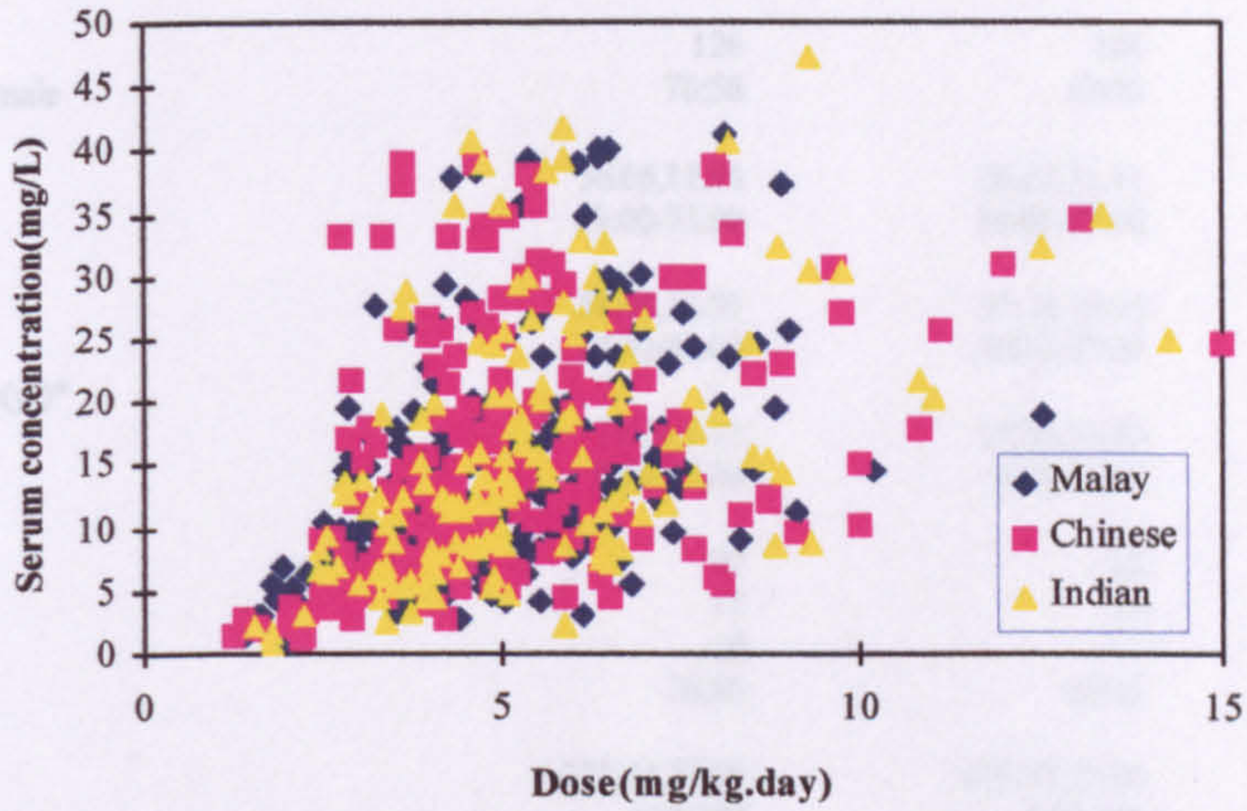


Figure 10- 3 : Plot of Clearance and dose in Malay, Chinese and Indian patients

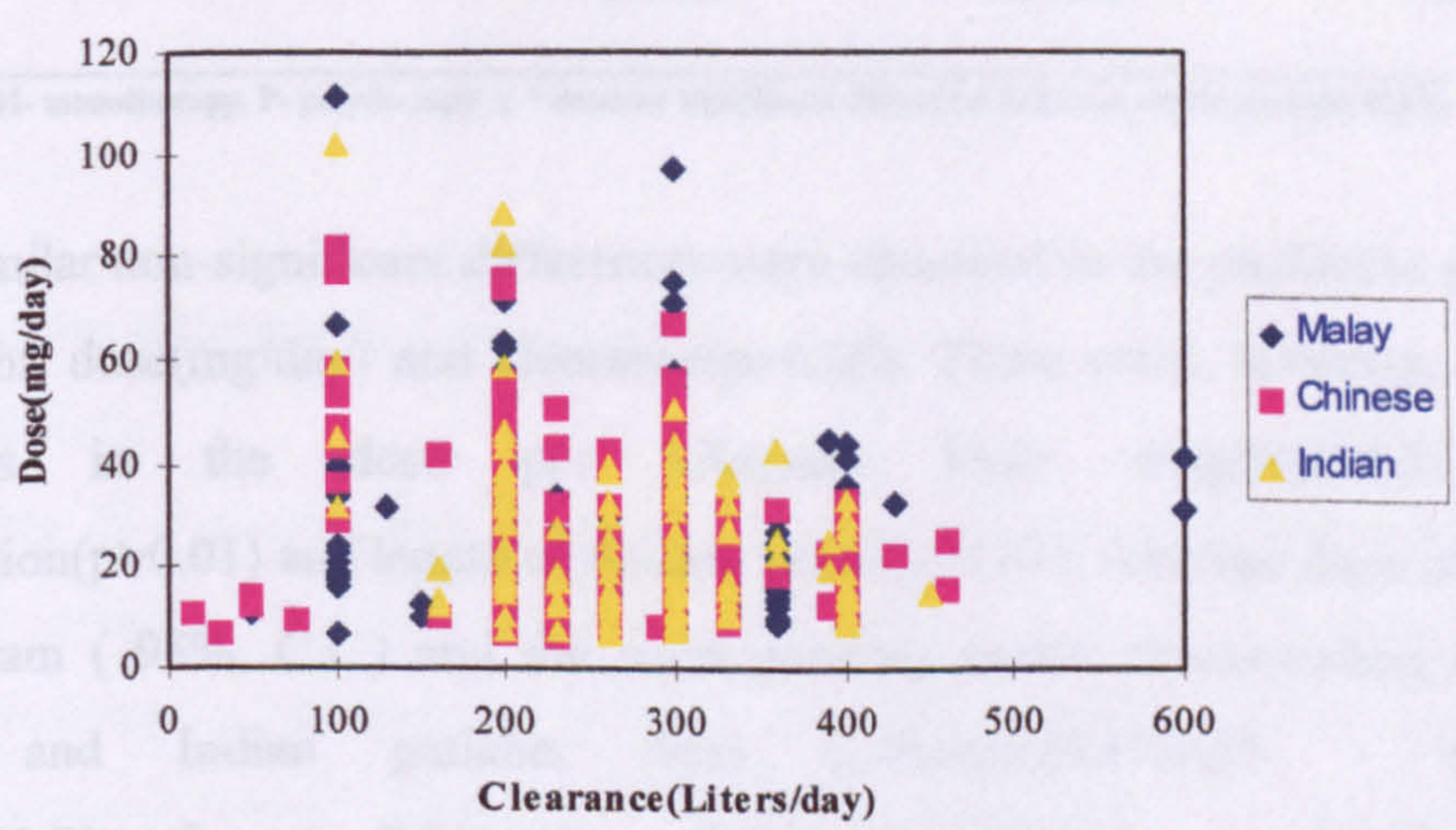


Table 10-2: Summary data of adult patients of various ethnic groups

Characteristic	Malay	Chinese	Indian
No. of patients	126	108	73
Ratio of male/female	70:56	53:55	35:38
Age(yr)			
Mean,sd	36.06,11.94	36.55,11.11	34.14,9.88
Range	19.00-73.00	19.00-69.00	19.00-53.00
Weight			
Mean,sd	58.94,10.91	59.58,10.92	57.06,10.84
Range	39.00-88.00	36.00-87.00	31.00-82.00
Disease duration(yr)*			
Mean,sd	11.21,9.03	15.08,11.89	15.65,10.42
Range	0.50-55.00	0.50-55.00	1.50-41.00
Epilepsy Types			
Generalised	99	82	58
Partial	17	18	9
Others	10	7	6
Therapy(M:P)	76:50	63:45	41:32
Dose(mg/day)			
Mean, sd	273.17,79.85	271.57,75.26	276.58,72.33
Range	100-600	100-460	100-450
Dose(mg/kg)			
Mean,sd	4.83,1.64	4.69,1.53	5.00,1.56
Range	1.39-10.17	1.39-11.11	1.55-9.76
Css(mg/L)			
Mean,sd	14.22,8.33	14.67,9.29	15.40,9.08
Range	0.90-41.29	1.23-38.93	0.89-40.62
CL(L/kg.day)			
Mean,sd	25.22,14.68	24.90,14.81	23.99,13.80
Range	7.25-111.11	5.91-81.30	6.62-102.04

Abbreviation: M- monotherapy, P- polytherapy ; * denotes significant difference between ethnic groups(<0.05)

Similar non-significant differences were observed in the paediatric patients for age, weight, dose(mg/day) and clearance($p>0.05$). There were, however, significant differences in the dose per kilogram body weight($p>0.05$), serum concentration($p>0.01$) and length of disease years($p>0.01$). Average dose in milligram per kilogram (95%, C.I) and the corresponding serum concentration for Malay, Chinese and Indian patients were 6.01mg/L(5.10mg/L - 6.92mg/L), 6.77mg/L(5.73mg/L - 7.81mg/L), 8.16mg/L(6.96mg/L - 9.35mg/L) and 11.59mg/L(9.95mg/L - 13.83mg/L), 13.23mg/L(10.46 - 16.00), 20.07mg/L(14.62mg/L - 25.52mg/L) respectively. These results clearly showed that Malay and Chinese patients were given distinctly lower doses per kilogram body weight than Indians which consequently produce lower serum concentration.

Mean length of disease years(95%,C.I) was again highest in Indian(11.57 years,10.18 years - 12.96 years) when compared with that of Malay (5.71 years,3.89 years- 7.53 years) and Chinese(5.92 years,4.56 years- 7.28years) patients.

Table 10-3: Summary data on paediatric patients of various ethnic groups

Characteristic	Malay	Chinese	Indian
No. of patients	11	18	11
Ratio of male/female	9:2	10:8	8:3
Age(yr)			
Mean,sd	12.77,4.22	12.42,4.01	14.09,2.37
Range	5.00-18.00	3.00-17.00	11.00-17.00
Weight			
Mean,sd	37.61,15.37	37.00,14.77	36.00,9.00
Range	11.75-56.00	12.00-60.00	26.00-56.00
Disease duration(yr)*			
Mean,sd	5.71,4.35	5.92,3.32	11.56,3.22
Range	1.50-14.00	2.00-17.00	3.00-15.00
Epilepsy Types			
Generalised	7	10	11
Partial	0	5	0
Others	4	3	0
Therapy(M:P)	3:8	10:8	6:5
Dose(mg/day)			
Mean, sd	212.27,80.35	242.64,105.90	277.73,73.35
Range	45-330	15-400	200-400
Dose(mg/kg)			
Mean,sd	6.01,2.18	6.77,3.18	8.16,2.86
Range	3.57-12.50	1.25-15.00	3.92-14.28
Css(mg/L)			
Mean,sd	11.59,5.36	13.23,8.49	20.07,13.04
Range	4.59-24.86	1.37-34.71	2.07-47.20
CL(L/kg.day)			
Mean,sd	20.43,9.33	22.61,14.45	21.52,18.11
Range	3.44-43.57	6.56,66.82	7.10-88.11

Abbreviation: M- monotherapy, P- polytherapy; *, significant difference between ethnic groups

10.3.1 Factors influencing *Km* and *Vmax*

a. Ethnicity

Table 10-4 summarises the pharmacokinetic characteristics in both adult and paediatric patients. Mean estimated *Km*(95%, C.I) for adult Chinese epileptics(6.49mg/L, 5.92mg/L - 7.06mg/L) showed a significantly lower value than those for Malay(8.54mg/L,7.81mg/L - 9.27mg/L) and Indian(7.47mg/L,6.71mg/L - 8.23mg/L) patients. The difference between Malay and Indian adult patients was not significant($p > 0.05$).

Inter-ethnic differences in *Km* were not observed in children($p = 0.22$). The mean(C.I,95%) values(mg/L) for Malay, Chinese and Indian patients were 9.56mg/L(7.08mg/L-12.04mg/L), 7.02mg/L(5.11mg/L-8.93mg/L) and 9.78mg/L(7.22mg/L-12.34mg/L) .

Table 10-4: Estimates of pharmacokinetic variables for various ethnic groups

Parameters	Malay	Chinese	Indian	p value	Conclusion
1. Children					
n	11	18	11		
Mean *Km*, sd (mg/L)	9.56,4.19	7.02,4.13	9.78,4.33	0.22	Homogenous
C.V(%)	43.83	58.83	44.27		
Mean *Vmax*, sd (mg/day)	366.40,114.70	383.20,152.00	438.90,94.40	0.31	Homogenous
C.V(%)	31.30	45.62	21.51		
Mean *Vmax*, sd (mg/kg.day)	11.05,4.99	10.97,4.84	12.86,4.21	0.18	Homogenous
C.V(%)	45.16	44.12	32.74		
2. Adult					
n	126	108	73		
Mean *Km*, sd (mg/L)	8.54,4.18	6.49,3.01	7.47,3.33	<0.01	Heterogenous
C.V(%)	48.95	46.38	44.58		
Mean *Vmax*, sd (mg/day)	472.30,157.50	432.40,98.00	440.00,103.60	0.04	Heterogenous
C.V(%)	33.34	22.66	23.55		
Mean *Vmax*, sd (mg/kg.day)	8.30,3.22	7.54,2.23	7.95,2.32	0.11	Homogenous
C.V(%)	38.80	29.76	29.18		
3. Pooled (Adult +children)					
Km,se (mg/day)	8.62,0.36	7.02,0.28	7.66,0.37	-	Homogenous in all ethnic groups
Q	0.60	0.27	2.87		
p value	0.38	0.67	0.06		
Vmax, se (mg/day)	457.33,13.02	429.21,13.02	439.101,11.18	-	Heterogenous for Malay, others homogenous
Q	8.05	1.76	0.001		
p value	<0.01	0.12	11.21		
Vmax, se (mg/kg.day)	8.39,0.28	7.66,0.21	8.17,0.27	-	Heterogenous for all ethnic groups
Q	3.22	8.73	14.31		
p value	0.04	<0.01	<0.01		

The coefficient of variation for *Km* in children and adult patients range between 32%-45% and 33-58%. These values clearly indicate a high interindividual variation of *Km* estimates for all ethnic groups.

The difference in *Km* values between children and adult patients for each ethnic group was investigated by pooling their weighted mean values and using the Q statistic to determine association. Pooled results for *Km*(95%,C.I) for Malay, Chinese and Indian patients of 8.62mg/L(7.91mg/L-9.33mg/L), 7.02mg/L(6.47mg/L-

7.57mg/L) and 7.66mg/L(6.93mg/L-8.38mg/L) revealed that the *Km* values of children and adult were not statistically different.

Vmax estimates in mg/day and mg/kg.day for paediatric patients of Malay, Chinese and Indian origin did not indicate the presence of any inter-ethnic difference. Significant differences of *Vmax* in mg/day were nevertheless observed for adult patients. *Vmax*(95%,C.I) of Malay(472.30mg/day, 444.80mg/day-499.80ma/day), Chinese(432.40mg/day, 413.92mg/day-450.88mg/day) and Indian(440.00mg/day, 416.23mg/day-463.77mg/day) showed the presence of inter-ethnic differences between Malay/Indian and Chinese adult epileptics. Differences between Malay and Indian patients were insignificant.

The coefficient of variation of *Vmax*(mg/day and mg/kg.day) in paediatric and adult patients were between 32%-58% and 22%- 46%. These values again highlight a high degree of interindividual variation in Malay, Chinese and Indian patients.

Pooled values of *Vmax*(mg/day and mg/kg,day) for adult and paediatric Malay epileptics were found to be different and statistically significant. Chinese and Indian patients only showed a similar significant difference when *Vmax* was measured in mg/kg.day. The present results show that *Vmax*(mg.kg.day) in children were higher than adults in all ethnic groups.

b. Length of disease years and type of epilepsy.

The effect of length of disease years and type of epilepsy on *Km* and *Vmax* were determined by dividing the adult and paediatric patients into several groups according to the number of disease years or the type of epilepsy. Altogether there were 7 groups of disease years ranging between less than 5 to more than 25 years. The three types of epilepsy were group as generalised, partial and those not classified into the earlier two groups.

Analysis of variance demonstrated the p values for the relationship between *Km*(mg/L) and *Vmax*(mg/day and mg/kg.day) and the length of disease in adult and children were statistically insignificant(Table 5a,5b). Linear regression of *Km*, *Vmax*(mg/day and mg/kg.day) also showed a poor correlation. The equations which portrayed the relationship are as follows;

I. Children

$$Km(\text{mg/L}) = 7.54 + 0.13(\text{Eyrs})$$
$$(r_{adj} = 0.0\%, p = 0.39)$$
$$Vmax(\text{mg/day}) = 350 + 5.93(\text{Eyrs})$$
$$(r_{adj} = 2.3\%, p = 0.18)$$
$$Vmax(\text{mg/kg.day}) = 11.3 + 0.03(\text{Eyrs})$$
$$(r_{adj} = 0.0\%, p = 0.85)$$

where Eyrs signify length of epilepsy disease years(years).

II. Adult

$$Km = 8.20 - 0.0438(\text{Eyrs})$$
$$(r_{adj} = 1.3\%, p = 0.03)$$
$$Vmax(\text{mg/day}) = 462 - 0.797(\text{Eyrs})$$
$$(r_{adj} = 0.1\%, p = 0.25)$$
$$Vmax(\text{mg/kg.day}) = 8.15 - 0.016(\text{Eyrs})$$
$$(r_{adj} = 0.1\%, p = 0.28)$$

These results proved that length of disease did not alter either *Km* and *Vmax* values in adult and paediatric patients.

Table 10-5a: Influence of Length of Disease and Types of Epilepsy on phenytoin pharmacokinetics in paediatric epileptics

Variables	**n**	***Km*(mg/L)**	***Vmax*(mg/day)**	***Vmax*(mg/kg/day)**
i.Length of disease				
< = 8 years	18	6.41,0.93	308.65,57.76	9.73,0.90
>8 - <12 years	6	6.14,2.28	333.65,90.48	10.41,3.85
12 - < 15 years	14	8.55,1.51	411.21,27.94	11.24,1.06
15 - <= 18 years	2	6.27,10.63	443.59,57.64	9.16,1.81
p values		0.51	0.44	0.76
ii. Type of epilepsy				
Generalised	28	7.79,0.96	375.70,29.42	10.90,0.78
Partial(focal)	5	4.24,1.24	367.82,120.38	9.62,2.35
Others	7	6.71,1.67	261.02,123.23	8.67,2.01
p-values		0.10	0.26	0.37

Results: mean ,sd

The findings for both age groups similarly revealed that the differences between *Km* and *Vmax* in relation to type of epilepsy were poorly correlated(mg/day

or mg/kg.day). These showed that type of epilepsy have no significant influence on both of these Michaelis-Menten parameters.

Table 10-5b: Influence of Length of Disease and Types of Epilepsy on phenytoin pharmacokinetics in adult epileptics

Variables	**n**	***Km*(mg/L)**	***Vmax*(mg/day)**	***Vmax*(mg/kg/day)**
i.Length of disease				
<5 years	61	7.34,0.51	435.46,14.32	7.52,0.26
5 - <10 years	74	6.67,0.42	453.09,14.25	7.66,0.30
10 - < 15 years	64	6.90,0.46	434.68,14.21	7.75,0.29
15 - < 20 years	28	7.23,0.77	430.52,20.96	7.95,0.41
20 - < 25 years	27	5.80,0.60	448.18,17.05	7.65,0.46
> 25 years	52	6.38,0.68	422.08,13.92	7.25,0.29
p values		0.34	0.69	0.80
ii. Type of epilepsy's				
Generalised	240	6.66,0.22	438.96,7.21	7.65,0.15
Partial(focal)	44	7.15,0.56	424.28,14.18	7.21,0.30
Others	23	6.74,0.87	443.15,25.25	7.64,0.58
p-values		0.70	0.68	0.48

Results: Mean, sd

c. Sex and therapy

Tables 10-6a and 10-6b presented the differences between *Km*(mg/L) and *Vmax*(mg/day and mg/kg.day) in relation to sex and therapy in both age groups. The results on children showed the corresponding p values for F test between *Km* and *Vmax* to sex and therapy were greater than 0.05. These values indicate that the estimated pharmacokinetic parameters in children were not significantly affected by sex and therapy.

Table 10-6a: Influence of Sex(male or female) and Therapy on phenytoin pharmacokinetics of paediatric epileptics.

Variables	**n**	***Km*(mg/L)**	***Vmax*(mg/day)**	***Vmax*(mg/kg/day)**
I. Sex				
male	27	9.36,4.11	369.10,138.10	11.39,4.65
female	13	6.65,4.27	445.40,90.90	11.77,4.89
p values		0.06	0.08	0.82
ii. Therapy				
Monotherapy	19	7.50,4.70	408.00,101.40	11.58,4.53
Polytherapy	21	9.37,3.81	381.10,150.70	11.45,4.91
p-values		0.17	0.52	0.93

Results: mean, sd

Km(mg/L) and *Vmax*(mg/day) in adult were similarly unaffected by the patient's sex or type of therapy. The correlation between *Vmax* measured in mg/kg body weight and sex was however highly significant in adult patients($p < 0.01$). Result highlighted that female adults have a significantly higher *Vmax*(mg/kg body weight) than that of male adults.

Table 10-6b: Influence of Sex(male or female) and Therapy on phenytoin pharmacokinetics of adult epileptics.

Variables	n	*Km*(mg/L)	*Vmax*(mg/day)	*Vmax*(mg/kg.day)
I. Sex				
male	158	7.54,3.71	453.30,105.80	7.42,2.14
female	149	7.59,3.72	447.70,148.10	8.50,3.12
p values		0.92	0.71	<0.01
ii. Therapy				
Monotherapy	184	7.44,3.62	445.90,132.20	7.82,2.86
Polytherapy	123	7.75,3.84	457.50,121.40	8.14,2.48
p-values		0.48	0.44	0.32

Results: mean, sd

d. Age

Adult and paediatric patients were categorically divided into 7 and 4 age groups respectively(table 10-7). The range in the lowest age group(8 years or less) for paediatric patients were wider due to a small number of patients within the age group.

Km(mg/L) was not significantly affected by age increases in either adult($p = 92$) or paediatric($p = 0.31$) patients. *Vmax* in mg/day and mg/kg.day in adult similarly showed a poor correlation with age although the latter did show a decreasing trend with respect to age.

Figure 10-4: Relationship of Vmax and age in paediatric patients

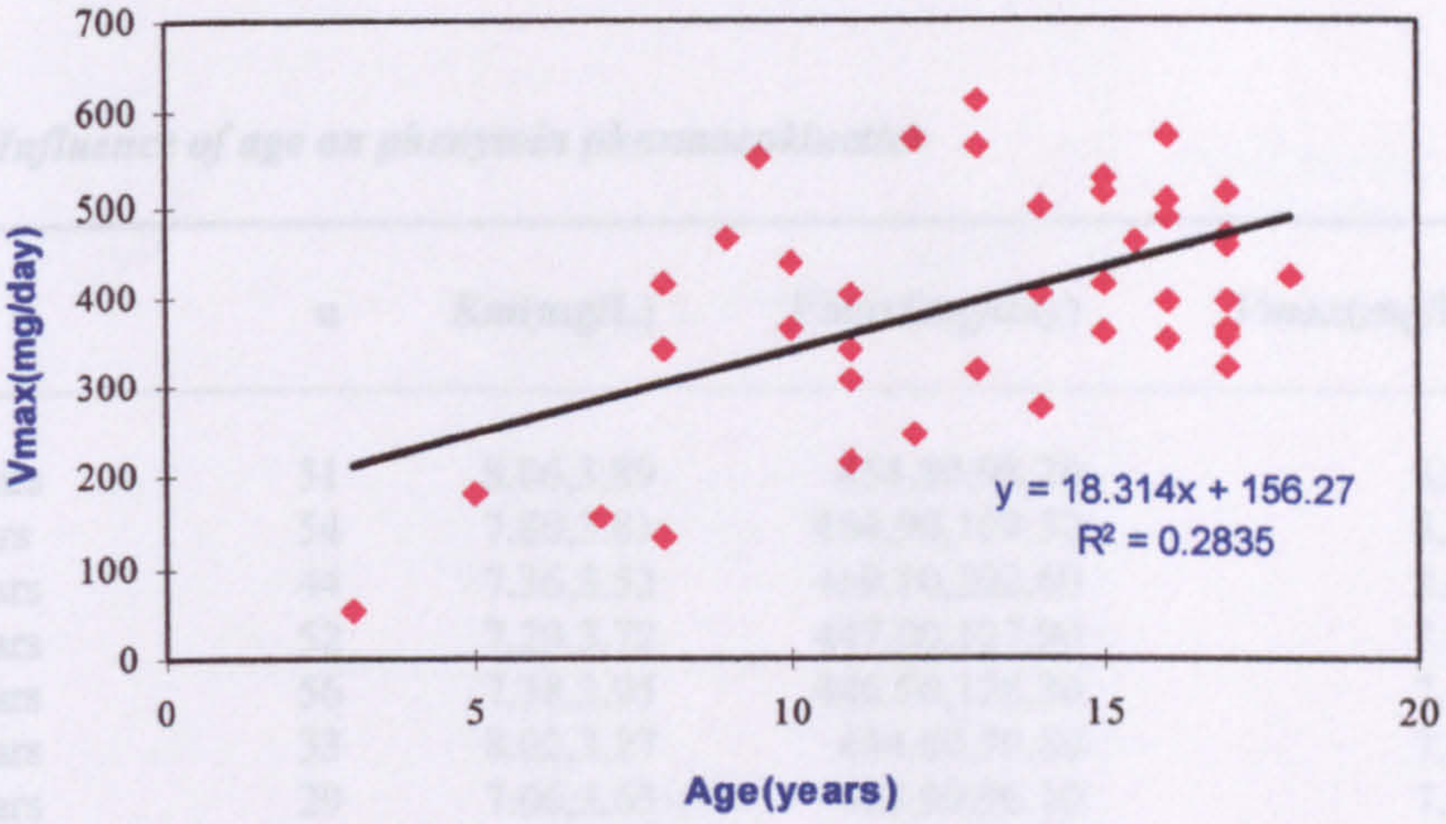
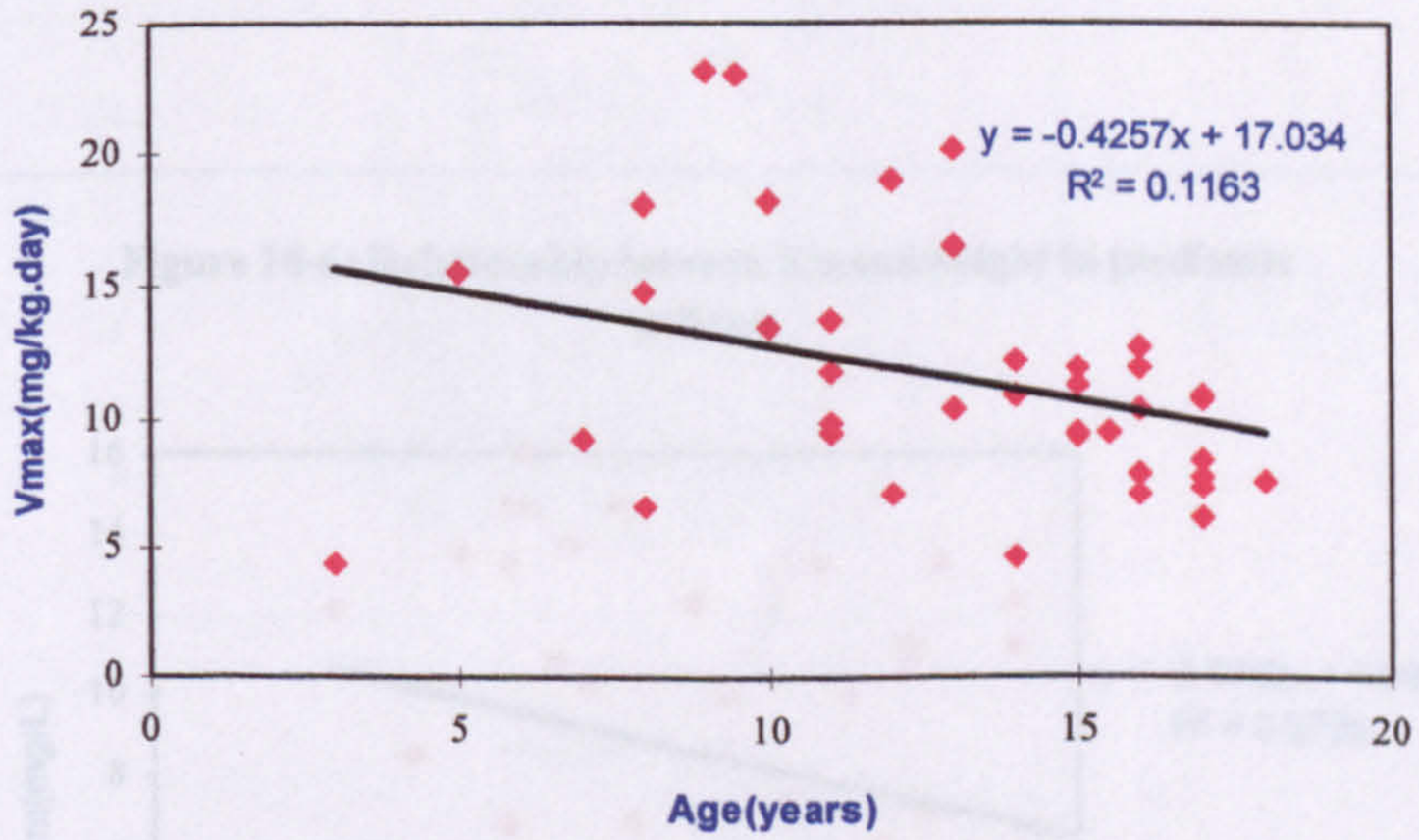


Figure 10-5: Relationship between Vmax(mg/kg) and age in paediatric patients



Evidence however showed that the values of *Vmax* in mg/day and mg/kg.day in paediatric patients differed significantly with age. Linear regression between *Vmax*

in both in mg/day(r_{adj} = 26.5% , p<0.01) and mg/kg.day(r_{adj} = 9.5%, p= 0.03) with age nevertheless showed the relationship were weakly related(figure 10-4, 10-5).

Table 10-7: Influence of age on phenytoin pharmacokinetics

Variables	**n**	***Km*(mg/L)**	***Vmax*(mg/day)**	***Vmax*(mg/kg.day)**
i.Adult				
>18- <23 years	31	8.06,3.89	454.80,98.20	8.65,2.47
23 - <28 years	54	7.80,3.81	454.90,109.50	8.22,1.96
28 - < 33 years	44	7.36,3.52	469.10,202.60	8.66,4.46
33 - < 38 years	52	7.29,3.72	447.00,127.90	7.73,2.68
38 - < 44 years	56	7.38,3.95	446.90,128.30	7.34,2.13
44 - < 49 years	33	8.02,3.27	444.60,70.50	7.51,1.91
49 - < 63 years	29	7.06,3.63	438.90,96.10	7.85,2.43
>63 years	8	8.24,4.41	418.50,133.60	7.23,2.43
p values		0.92	0.97	0.17
ii. Children				
< 8 years	6	9.65,4.45	213.20,136.70	11.43,5.49
8 - < 12 years	8	10.56,4.65	386.30,102.50	15.35,5.49
12 - < 15 years	8	7.03,4.79	436.50,143.20	12.59,5.55
15 - ≤ 18 years	18	7.81,3.79	438.60,72.30	9.35,1.93
p values		0.31	<0.01	0.02

Results: mean, sd

Figure 10-6: Relationship between Km and weight in paediatric patients

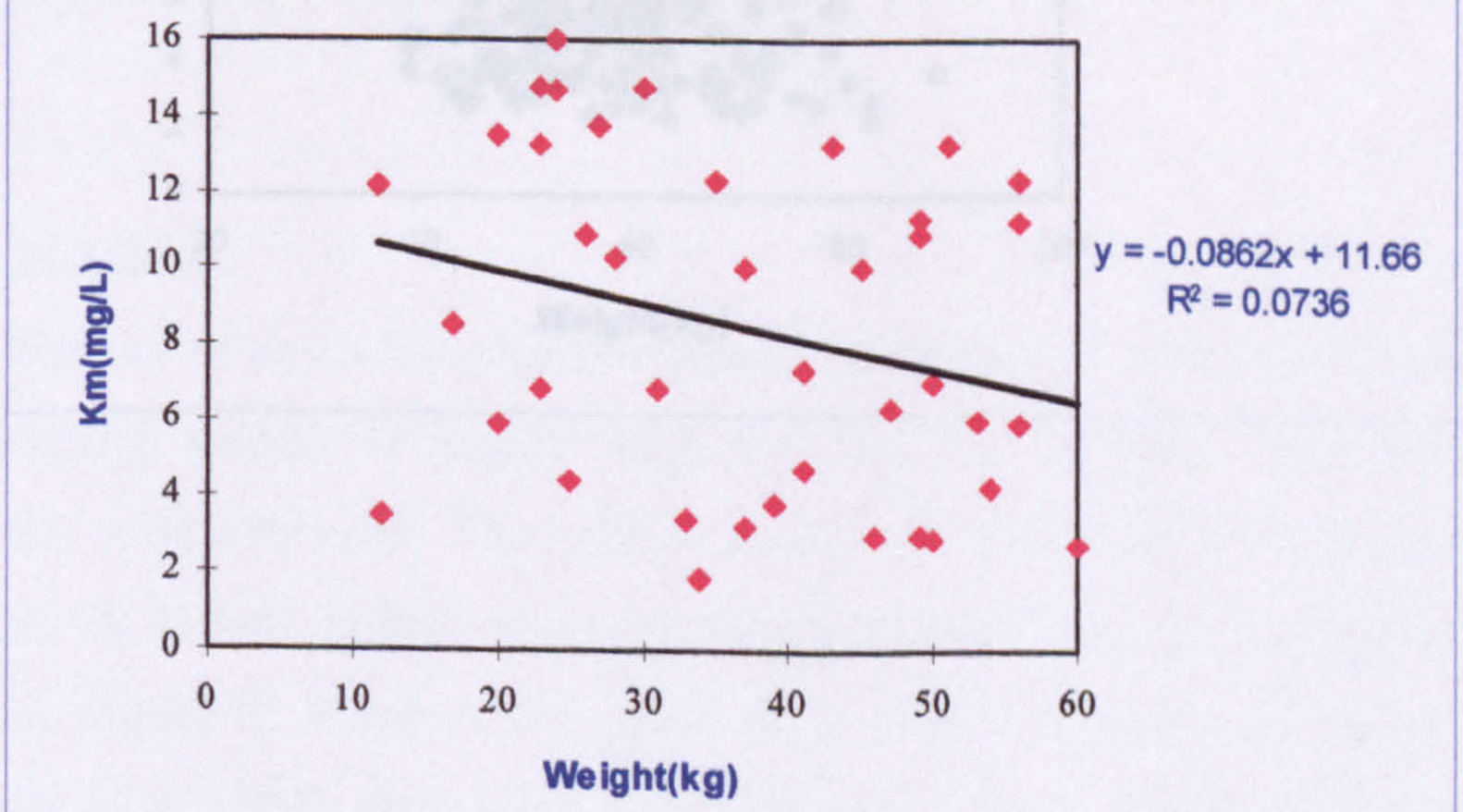


Figure 10-7: Relationship between Vmax and weight in paediatric patients

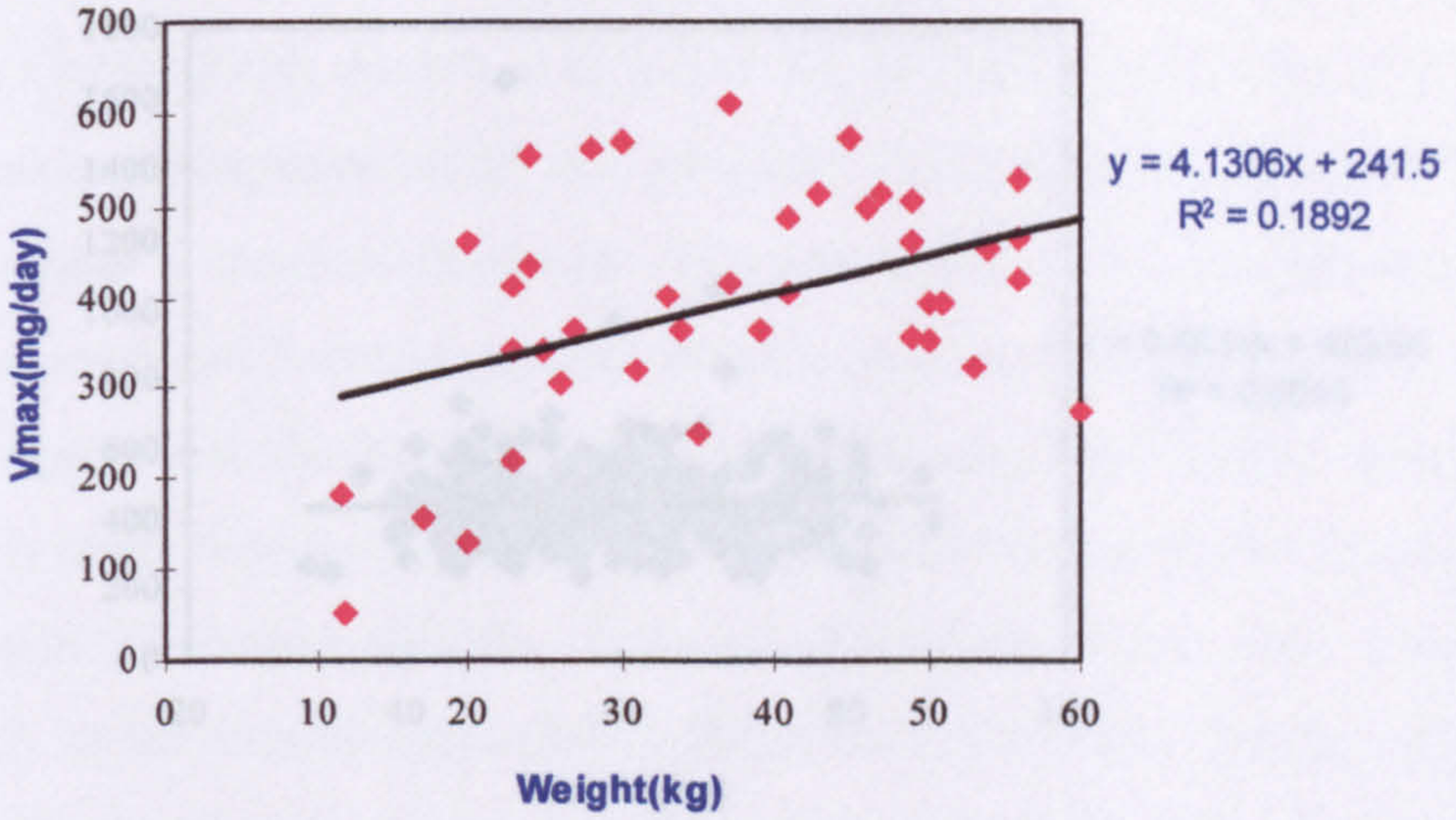


Figure 10-8: Relationship between Km and weight in adult patients

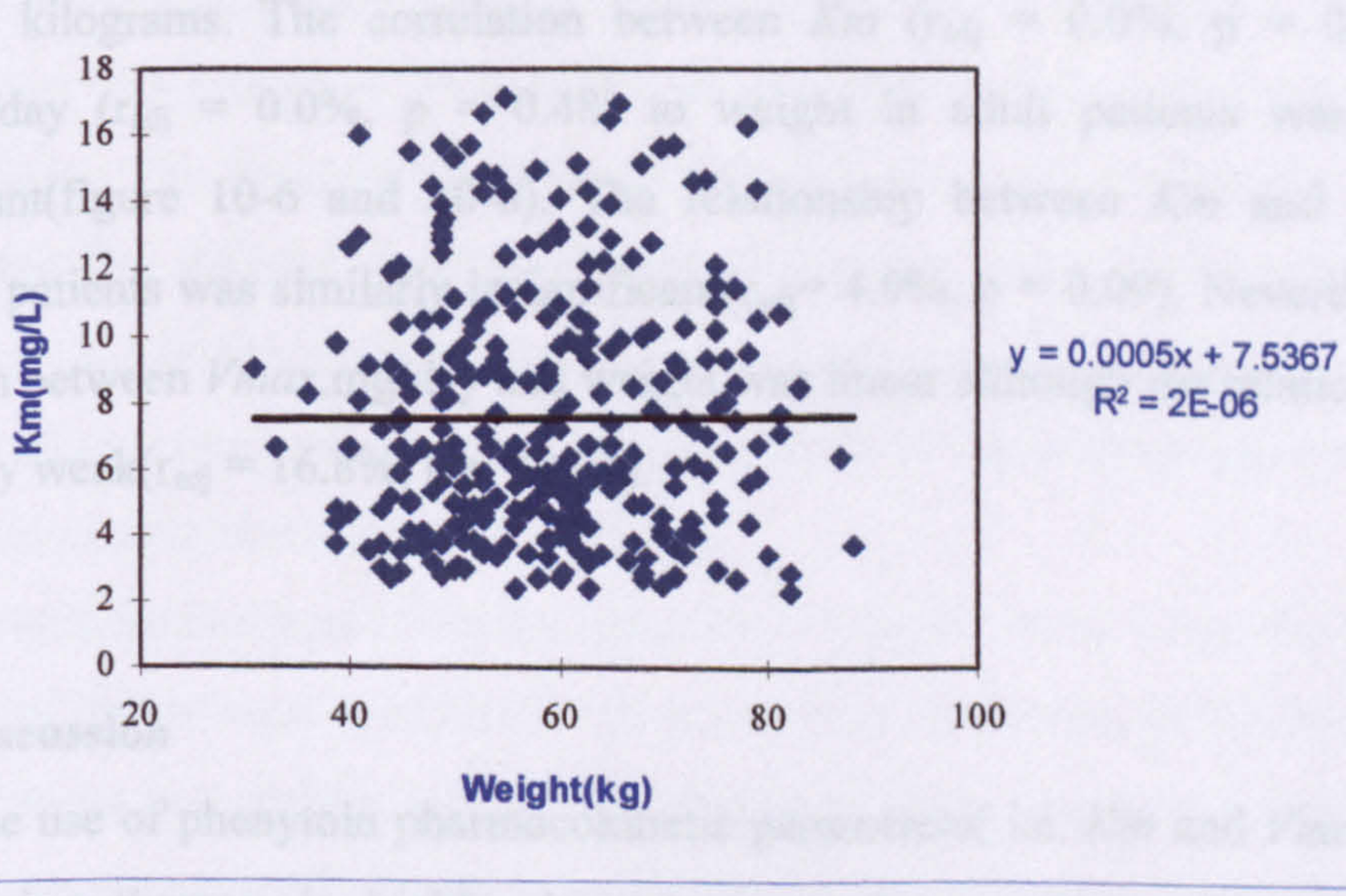
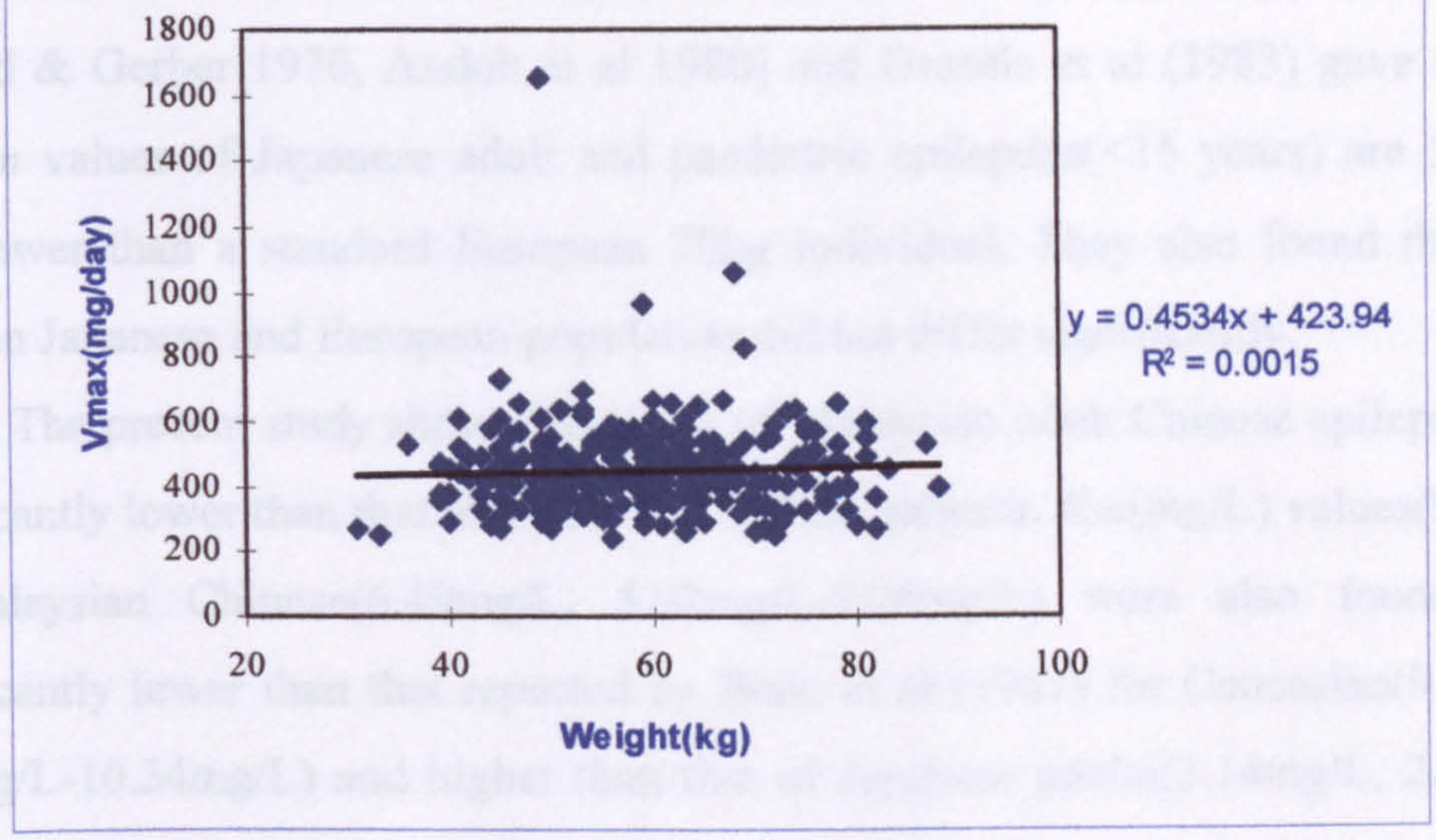


Figure 10-9: Relationship between Vmax and weight in adult patients



e. Weight

Linear regression was used to determine the relationship of *Km* and *Vmax* to weight in kilograms. The correlation between *Km* (r_{adj} = 0.0%, p = 0.93) and *Vmax*,mg/day (r_{adj} = 0.0%, p = 0.48) to weight in adult patients was low and insignificant(figure 10-6 and 10-8). The relationship between *Km* and weight in paediatric patients was similarly insignificant(r_{adj}= 4.9%, p = 0.09). Nevertheless, the correlation between *Vmax*,mg/day and weight was linear although the relationship was statistically weak(r_{adj} = 16.8%, p = <0.01).

10.4 Discussion

The use of phenytoin pharmacokinetic parameters(i.e. *Km* and *Vmax*) for use in optimizing therapy is highly dependent on the accuracy with which these parameters were estimated. The present method of estimating these parameters has been used by various authors in determining population-specific pharmacokinetics in European [Bauer & Blouin 1983, Blain et al 1981], Japanese [Chiba et al 1980, Watanabe et al 1997] and Asian [El-Sayed et al 1989, Ismail & Rahman 1990] population. Results of these studies have also showed the accuracy of the estimated

serum concentration to that of observed concentrations [El-Sayed et al 1989, Chiba et al 1980].

Genetic differences have been shown to influence phenytoin pharmacokinetics [Arnold & Gerber 1970, Andoh et al 1980] and Grasela et al (1983) gave evidence that *Km* values of Japanese adult and paediatric epileptics(<15 years) are 57% and 67% lower than a standard European 70kg individual. They also found that *Vmax* between Japanese and European population did not differ significantly.

The present study showed that *Km* of Malaysian adult Chinese epileptics were significantly lower than that of Malay and Indian patients. *Km*(mg/L) values(95%,C.I) of Malaysian Chinese(6.49mg/L, 5.92mg/L-7.06mg/L) were also found to be significantly lower than that reported by Blain et al (1981) for Caucasian(9.40mg/L, 8.43mg/L-10.34mg/L) and higher than that of Japanese adults(3.14mg/L, 2.22mg/L-4.06mg/L) [Watanabe et al 1997].

Km of Malay(8.54mg/L, 7.81mg/L-9.26mg/L) epileptics were found to be similar to Caucasian and significantly higher than Japanese. The current result for Malay adult epileptics was also similar to those reported by Ismail & Rahman 1990 on Malaysian Malay epileptics(7.45mg/L, 2.50mg/L-12.35mg/L) although a wider range of *Km* values was estimated. This difference may be due to the small sample size(9 patients) used. The findings on Indians(7.47mg/L, 6.71mg/L-8.23mg/L) epileptics revealed that they differed significantly from Caucasian and Japanese adult patients. Thus, although *Km* values of Indian and Malay epileptics were similar, differences with other ethnic population do exist and care should be taken when these patients are to be treated in countries outside Malaysia.

Results for children similarly showed that Chinese have lower *Km* value than Malay or Indian patients. Statistically, there were no interethnic differences between Malay, Chinese and Indian paediatric epileptics. These findings should be taken cautiously since the small sample size can lead to the observed high interindividual variation and may mistakenly portrayed the current homogeneity between them. However, comparing *Km* values of the current population to those of Japanese and Caucasian are suggestive of interethnic differences.

The mean *Km* in mg/L(95%,C.I) of Malaysian paediatric population(8.44mg/L, 7.10mg/L-9.77mg/L) is definitely higher than Japanese [Chiba

et al 1980, 3.70mg/L(2.88mg/L-4.52mg/L)]. Reports for Caucasian population showed that the current population was similar to those reported by Blain et al (1981)(7.50mg/L, 5.07mg/L-9.95mg/L) but significantly different from those of Bauer et al (1983)(6.26mg/L, 6.08mg/L-6.45mg/L). These differences could be attributed to the former using a population age of less than 8 years old while the latter age range was between 0.5 to 16 years. Since the present study employed an age range of 2.5 to 18 years, the current results did show that Malaysian paediatric population have a higher *Km* than Caucasian. Results of Arab paediatric patients [El-Sayed et al 1989, 7.75mg/L(6.45mg/L-9.04mg/L)] nevertheless showed that Malaysian and Arab population were statistically similar.

The *Vmax* in mg/kg.day of Malaysian Chinese, Malay and Indian adult and paediatric patients were found to be similar. Mean *Vmax*(95%,C.I) of Malaysian adult population(7.95mg/kg.day,7.64mg/kg.day-8.25mg/kg.day) were found to be statistically higher than that of Japanese [Watanabe et al 1997,(5.34mg/kg.day, range 4.67-6.01 mg/kg.day)] and similar to that of Arabs [El-Sayed et al 1989 (6.91mg/kg.day, range 6.13-7.69mg/kg.day)] and Caucasian population (8.70mg/kg.day,range 7 31-10.09 mg/kg.day).

The current results however revealed the estimated *Vmax* of Malay epileptics[8.30mg/kg.day,7.73mg/kg.day-8.86mg/kg.day] were similar to that reported by another Malaysian author [Ismail & Rahman 1990, (8.62mg/kg.day,(7.80mg/kg.day-9.44mg/kg.day)]. Vmax measured in mg/day in adults showed that Malay patients(472.30mg/kg.day,452.97mg/kg.day-486.63mg/kg.day) have a significantly higher values than that of Chinese(432.40mg/kg.day,413.92mg/kg.day-450.88mg/kg.day). These results could indicate that the relative size of liver per body weight is larger in the Malay population which then demonstrated by the higher metabolic capacity [Bauer & Blouin 1983].

Vmax for children also showed that there were no inter-ethnic differences between Malay, Chinese and Indian epileptics. The mean *Vmax*(95%,C.I) in mg/kg.day of Malaysian paediatric population of 11.51mg/kg.day(10.06mg/kg.day-12.96mg/kg.day) were different to that of Japanese [Chiba et al 1980, 9.54mg/kg.day(9.12mg/kg.day - 9.95mg/kg.day)] and similar to that reported for

Caucasian [Bauer et al 1983, 10.22mg/kg.day(5.77mg/kg.day - 14.66mg/kg.day)] and Arabs [El-Sayed et al 1989,10.93mg/kg.day(9.40mg/kg.day-12.46mg/kg.day)].

Table 10-7: Studies that utilised the graphical method to estimate *Km* and *Vmax*

References	Ethnic group -sample size	Therapy	Age-group (range)	*Km* (mg/L) mean,sd	*Vmax* (mg/day) mean,sd	*Vmax* (mg/kg.day) mean,sd
1. Children						
Chiba et al (1980)	Japanese -104	Poly	0.5-16	3.7,0.42	256.00	3.54,4.30
Blain et al (1981)	Caucasian -40	Mono	< 8 years	7.5,1.24	238.00,25.50	20.4,2.07
Bauer et al (1983)	Caucasian -135	unstated	0.5-16	6.26,0.09	-	10.22,2.27
El-Sayed et al (1989)	Arabs -5	Mixed	2.5-17	7.75,0.66	234.00,49.06	10.93,0.78
Ismail & Rahman (1990)	Malay -5	Mixed	10-18	5.28,1.97	292.84,35.82	8.35,0.67
2. Adult						
Blain et al (1981)	Caucasian -21	unstated	18-66	9.4,2.26	542.20,37.00	8.70,0.71
El-Sayed (1989)	Arabs -12	Mixed	19-47	6.44,1.01	417.00,19.13	6.91,0.40
Ismail & Rahman (1990)	Malay -9	Mixed	19-43	7.45,2.50	454.84,44.76	8.62,0.42
Watanabe et al (1997)	Japanese -16	Poly	19-35	3.14,0.47	-	5.34,0.34
3.Adult + Children						
Rambeck et al (1979)	German -127	Mixed	6-72	6.01,3.71	355.00, 106.00	-
Lai (1985)	Chinese -50	Mixed	Both	4.00	range 245-562	-

Abbreviation: mono-monotherapy, poly-polytherapy, mixed-mixed monotherapy and polytherapy, unstated- therapy not mentioned

The result(*Vmax*,mg/kg.day) for Malay paediatric epileptics(11.05mg/kg.day,10.17mg/kg.day-11.92mg/kg.day) was different to an earlier report on Malaysian paediatric population by Ismail & Rahman 1990(8.35mg/kg.day,7.04mg/kg.day-9.66mg/kg.day). This difference can be attributed to the latter using an age range of 10-18 years while the present study utilising an age group of 2.5 - 18 years old. Another factor that could have affected the results of both study is the small sample size of only 11 and 5 patients respectively.

The above observations in differences in *Km* and *Vmax* among different ethnic groups can be attributed to the number of slow and extensive metabolizers in the population. Individuals are segregated as slow or extensive metabolizers on their ability to metabolise phenytoin to 4'hydroxy-phenytoin [Kutt et al 1964, Vasko et al 1980, Wolff et al 1983]. This differences in phenytoin hepatic metabolism or polymorphism is associated with the polymorphic enzyme called P4502C9 [Veronese et al (1993a), Veronese et al (1993b).

The incidence of the poor metaboliser phenotype in the Japanese population is reported to be approximately 0.2% [Watanabe et al 1997]. Values for Caucasian have been found to be 0.002% [Inaba T 1986] and 0.03% [Arnold & Gerber 1970]. These differences of slow phenytoin metabolisers among ethnic groups thus displayed the possibility for the inter-ethnic differences observed in the studied population.

Horsman et al (1997) later published findings in the existence of phenytoin genetic polymorphism in Caucasian. Their study reported the index for phenytoin hydroxylation in urine for 122 healthy Caucasian appear to be bimodally distributed with 92% of the population showing a mean(SE) value of 6.39 ± 0.099 and 8% a mean(SE) of 1.00 ± 0.18. They also found no apparent differences in phenytoin hydroxylation index values between males and females, between females taking and not taking oral contraceptives, smokers and non-smokers and drinkers and non-drinkers. Their final conclusion recommended that future studies on phenytoin pharmacokinetics should include P4502C9 genotyping in order to ascertain for genetic polymorphism.

These findings may explained why Malaysian Chinese have a significantly lower *Km* than Malay, Indian, Arabs or Caucasian population. Unfortunately, studies on phenytoin genetic polymorphism among Malaysian Malay, Chinese and Indian

population has yet to be reported. Nevertheless, based on the above observations, the proportion of slow metabolisers among ethnic groups and percentage of slow metabolisers in each ethnic groups(genetic polymorphism) could be the two important factors for the observed high inter-individual variation and inter-ethnic differences.

Houghton and Richens et al (1975) reported that factors such as age, weight, height and sex have little effect on the interindividual variation in serum phenytion concentration. They further pointed that adjustment of dosage by age, weight or height would only achieve marginal improvement. Their findings concluded that genetic differences and the effect of saturation kinetics are more important in determining steady-state concentrations. Based on these results, it is not surprising that very few authors quoted the impact of height, sex or weight on the pharmacokinetic parameters(*Km* and *Vmax*) of phenytoin. The current findings also provide further data that indicate that the influence of length of disease, type of epilepsy and types of therapy are also insignificant.

Nevertheless, a significantly higher *Vmax* per kilogram body weight obtained in female adult epileptics might indicate that sex does have an impact on the overall pharmacokinetics of phenytoin. This finding was similarly observed by Ismail & Rahman (1990) but not by Bauer et al (1982) and can be explained by the observations of Travers et al (1972) and Houghton and Richens (1975). These authors found that women had a lower mean serum concentration than men although the difference did not reach statistical significance. The result of the current study was similar($p = 0.62$) but the difference might be attributed to the weight(kg,standard error) of female(54.16kg,0.60) being significantly lower than male(63.01kg,0.60) patients($p = <0.01$).

The effect of age to *Km* has been reported to be insignificant in both adult and paediatric patients [Bauer et al 1982, Bauer et al 1983, Chiba et al 1980] and was similarly observed in this study. Reports on the linear relationship between *Vmax*(mg/kg.day) and age is quite established in the paediatric population [Chiba et al 1980($r = -0.55$), Bauer et al 1983($r = -0.55$)]. Similar association in the adult population has only been shown to be significant by Bauer et al (1982)($r = -0.52$)] although poor relationship have been noted by Sherwin et al (1974), Houghton and

Richen (1975) and Taylor et al (1983). Dodson (1982) published results that this relationship is highly correlated only after applying non-linear regression analysis.

The results of the current study found a similar but weak relationship for *Vmax* in mg/day(r_{adj} = 26.5%, p = <0.01) and mg/kg.day(r_{adj} = 0.1, p = 0.03) for the paediatric patients. Results for adults(*Vmax*,mg/kg.day) however showed that the relationship was absent although the age range was similar to that used by Bauer et 1982.

These results may be expected as the current retrospective study is not specifically designed to investigate these relationship although a trend towards linearity(adults, r_{adj} = 1.1% , p = 0.04) was observed. It should also be noted that the prospective study by Bauer et al (1982), although more controlled, displayed a similar and distinctively wide scatter along the regression line which thus indicate a high interindividual variation. The present study also comprises of a mixed population of Malay, Chinese and Indian patients which again might contribute to the lesser degree of significance.

Weight is observed to be poorly related to *Km* in both population groups and a similar result on the adult population has been reported by Bauer et al (1982). This study provided results for *Vmax* and weight which only showed a weak linear relationship (r_{adj} = 16.8%, p <0.01) in the paediatric population. El-Sayed et al (1989) and Ismail and Rahman (1990) nevertheless published results that the relationship in a mixed age population is linear. The observed result for this study for a mixed population group however displayed that this relationship is again poor although a trend towards linearity is demonstrated(r_{adj} = 2.1%, p < 0.01).

Theoretically, the observed linear relationship between *Vmax* with age and weight can be explained by phenytoin having a variable hepatic metabolism with increasing age. Mitsukawa (1967) relates these observation in children to changes in metabolic capacity(*Vmax*) with increased age. Metabolic capacity is shown to be dependent on the relative size of liver per body weight and the relative activity of drug metabolising enzyme per gram of liver [Bauer & Blouin 1983]. Thus, liver size per unit body weight decreases with age so that children have an increased relative hepatic size compared to adults.

The decline in *Vmax* with age in adults(especially the elderly) is described by Wood et al (1979) by alterations in drug metabolism which are related to changes in hepatic blood flow, liver enzyme content and response to environmental factors. Although phenytoin elimination is not limited by the amount of liver blood flow, the decrease in *Vmax* is probably reflected by age-dependent changes in the amount or efficiency of drug metabolising enzymes in the liver. Thus, with aging clearance is expected to decrease which then lead to lower *Vmax* in older patients which may explain the negative linear relationship with age.

10.5 Therapeutic implication

The results of this study provide evidence that inter-ethnic differences are present in the Malaysian population. Based on the resulting *Km* and *Vmax* values, the estimated baseline dose for initiation of therapy in order to achieve a serum concentration of 10 mg/L for adult can be calculated by the following formula described by Ludden et al (1977).

$$R = \frac{Vmax \,.\, Cpss}{Km + Cpss}$$

where *R* is the dosing rate(mg/day), *Vmax*, the maximum daily rate at which phenytoin is metabolised(mg/day) and *Km*(mg/L), is the elimination rate by which is half of *Vmax*. Substituting the population values of *Km* and *Vmax* of each ethnic group into the formula, the mean dose for Malay, Chinese and Indian epileptics are 254.7mg/day, 262.2mg/day and 251.9mg/day. Mean dose for the upper end of the therapeutic range (20mg/L) are 331.0mg/day, 326.5mg/day and 320.0mg/day. Similar estimates for Japanese(70kg individual) [Watanabe et al 1997], Caucasian [Blain et al 1981] and Arabs [El-Sayed et al 1989] are 284.5mg/day and 323.1mg/day, 279.5mg/day and 368.8mg/day, and 253.6mg/day and 315.4mg/day. These estimates showed that although there are differences in *Km* values, the final mean daily dose looks almost similar and seems clinically unimportant. These findings however highlight the fact that the baseline and maximum mean dose for adults of any ethnic group is between 200mg/day to 400mg/day. This would definitely help medical practitioners to titrate

the most effective dose for each individual in the fastest time possible and the maximum dose would help to determine as to when toxic symptoms would be suspected.

The corresponding values for the paediatric population to achieve similar concentration of 10mg/L and 20 mg/L are 180.7mg/day and 276.6mg/day. Values for Japanese [Chiba et al 1981], Caucasian [Blain et al 1981] and Arabs [El-Sayed et al 1989] are 186.9mg/day and 216.0mg/day, 78.3mg/day and 117.8mg/day, and 111.8mg/day and 151.1mg/day. These values clearly demonstrated that inter-ethnic differences population pharmacokinetics have an important role during initiation of therapy in paediatric patients.

10.6 Limitation of study

The present study suffers by not being able to establish the accuracy of the estimated *Km* and *Vmax* values of real patients. This is of clinical importance since the resultant population pharmacokinetic parameters are derived from data that were gathered retrospectively. Thus it is proposed that the observed Michaelis-Menten parameters be tested prospectively on patients of each ethnic groups so as to ascertain its true relevance in a clinical setting.

10.7 Conclusion

This study has provided evidence of the existence of inter-ethnic differences in phenytoin pharmacokinetic parameters (*Km* and *Vmax,mg/day*) among Malay, Chinese and Indian adults patients of Malaysia. Results for paediatric patients did not display similar findings.

Km which is a value equal to the plasma concentration at which the rate is one-half the maximum of Chinese was found to be lower and differed significantly to that of Malay and Indian adult patients. The differences between Malay and Indian adult patients were however insignificant. Maximum metabolic rate capacity (*Vmax,mg/kg.day*) of all three ethnic groups were statistically similar.

There was no indication of a linear relationship between *Km* with age and weight in both adult and paediatric patients. Relationship between *Vmax*(mg/kg.day)

with age or weight showed the inclination towards a linear relationship in paediatric patients only. These relationships were however statistically weak.

The mean dose for Malay, Chinese and Indian adult epileptics in order to achieve the minimum target concentration of 10mg/L range between 251.9 to 262.2mg/day (4.5mg/kg.day - 4.6mg/kg.day). The doses for the upper end of the therapeutic range(20mg/L) are between 320.0mg/day to 331.0mg/day (5.7mg/kg.day - 5.8mg/kg.day). The corresponding values for the paediatric population to achieve similar concentration of 10mg/L and 20 mg/L are 180.7mg/day(6.2mg/kg.day) and 276.6mg/day(8.1mg/kg.day).

Chapter 11

Overall discussion

Studies evaluating the impact of therapeutic drug monitoring (TDM) of antiepileptic drugs such as phenytoin, carbamazepine, valproic acid and phenobarbitone have demonstrated both significant [Wing & Duff 1989, Ioannides-Demos et al 1988] and insignificant improvement [Botha et al 1990, McFayden et al 1990] in seizure outcome. Based on these outcome studies, it is difficult to draw a general conclusion about its effectiveness since some patients or patient groups do benefit from TDM. These patients or patient groups need to be redefine since TDM based on data from different populations and indications provide no value in the provision in clinical care [Ensom et al 1998].

The impact of ethnicity on drug treatment has been shown to be clinically important in phenytoin but reports on carbamazepine, valproic acid and phenobarbitone have been rather inconclusive. The observations on phenytoin are however based mainly on reports by researchers from the developed countries while those from the developing world are few. Defining phenytoin ethnic-specific pharmacokinetic parameters is thus difficult and any attempt to extrapolate these data onto other ethnic groups might be clinically and therapeutically inappropriate. Similarly the few reports on the other three antiepileptic drugs have yet to be verified and the current thesis has objectively undertaken to investigate the impact of ethnicity on the therapeutic ranges and pharmacokinetics of these drugs in its current treatment practise.

Quantitative evaluation of the influence of ethnicity on established antiepileptic drugs such as phenytoin, carbamazepine, valproic acid and phenobarbitone is restricted by the relatively limited number of published studies that disclosed the ethnicity of their studied subjects. Of those where the ethnicity was mentioned, differences in inter-study variation such as study design and /or pharmacokinetic models further hindered the possibility of either identifying, verifying or substantiating the existence of inter-ethnic differences.

The current use of retrospectively collected data has always been deemed inferior to that of prospective study design. Problems of reliability, bias and improper study design

do restrict its overall use. However, this method is suitable and applicable within the limits of this study set of objectives. The present study aim to examine 'real' patients from a clinical setting of a hospital. This is of clinical importance since the absence of restriction for study admittance of patients groups provide both typical, atypical and extreme results which controlled study designs find difficult to construct. Routine data is easily available as well as cheap and less time consuming to conduct. This type of data do have problems of extraneous factor or 'noise' but these so called variability may suggest that further exploration may be valuable [Sheiner et al 1977].

Disparities in the inclusion and exclusion criteria defined in this study are not entirely absent. The most prominent setback of routine data is the belief that the written patients' notes were correct. Studies by Mckee et al (1993) and Schoenenberger et al (1995) have showed that the percentage of non-compliance to drug therapy of these drugs contributed to about 1 to 9 percent of the total number of requests for serial monitoring of these drugs. Based on these findings, to assume full confidence in the written information(patients notes) may be too idealistic. However, by admitting a large number of patients(1,215) into the study and defining the 95% confidence interval of each conclusion have manage to improve the degree of statistical strength of the findings.

The current study has set 30 days for the baseline for the drugs to reach steady-state and is in-line with published data [Prescott 1980]. However, the lack of information in the studied population(Malay, Chinese, Indians) period for carbamazepine autoinduction to complete could raise some concern to the appropriateness of the set baseline period for admission. This point is not entirely unfounded since reports of the completion of autoinduction from two Asian countries such as Korea [Yoon et al 1996] and Taiwan [Lin et al 1991], have been shown to be 1.5 times longer and significantly different than in Caucasians. The effect of the possible differences in the time for completion of autoinduction on the present study may well be insignificant since most of the patients were on the same dose regime for more than 90 days and this time period is definitely higher than that is reported of 30 to 45 days.

Differences in the bioavailability factor might also affect the final conclusion of this study. This information is important in the current study since bioavailability may varies between ethnic groups or products. The is another area where many developing nations such as Malaysia overlook since information given by the drug companies are

taken to be applicable to the local population. Diet is another factor that is known to affect bioavailability and inter-ethnic differences in diet could play an important role in the present study. Differences in bioavailability among ethnic groups could also contribute to the observed high inter-individual variation.

The present study is however able to achieve its set objectives by utilising a total of 1215 patients data of Malay, Chinese and Indian origin in order to satisfy the population-based methodology implemented in the analysis. The advantageous of the current population analysis over individual analysis has been well-defined by Hashimoto & Sheiner (1991) and described in chapter 3 section 3.2.1. In summary, population analysis perform as well as individual analysis if the data is plentiful (in this study the smallest number of patients data(190)) and observational errors due to poor study design (as described above) in population analysis is better control than individual analysis. The latter is described by Hashimoto & Sheiner (1991) due the ability of the population analysis being able to exploit all informations of patients data simultaneously.

The linear model employed in this study is the simplest pharmacokinetic model. It predicts that effects (in this study, i.e, serum concentration or clearance) are directly proportional to the dose. The advantage of this model rests in that parameter estimation is easily performed using linear regression. The main disadvantage is however it cannot predict a maximum effect outside or above those which were used to estimate the parameters. Nevertheless, since the main objective of the current population study is to investigate for variation in effects due to variables such as ethnicity, age, weight etc, the slope of the regression line provides the best technique to observe for differences.

The present study reviewed literature on the issue of inter-ethnic differences and found statistical evidences of inter-ethnic difference in the population pharmacokinetics of phenytoin in both individual and population base studies. Published reports on comparative studies showed that phenytoin clearances between Eskimos, Blacks and Caucasian were significantly different. Grasela et al 1983 reanalysed data on reported Japanese and Caucasian population and found that *Km* values of Japanese to be fifty-seven percent lower than Caucasian. Individual and population based studies similarly showed the mean differences in pharmacokinetic parameters(clearance, *Km* and *Vmax*) can be more than 1.5 fold among ethnic groups such as Caucasian, Japanese, Chinese, Arabs, Blacks and mixed Malaysian population.

This study also showed that there are inter-ethnic differences of phenytoin population pharmacokinetics among Malaysian Malay, Chinese and Indian patients. Mean *Km* and *Vmax(mg/day)* values for adult Malay and Indian patients are statistically different to that of Chinese patients. These differences are however not observed in younger patients although this may be associated with the relatively small number of patients in each age group. The present study also showed that the range for inter-individual variation for *Vmax* (adult : 29.2% - 38.8%, children : 32.7% - 45.2%) and *Km* (adult : 44.6% - 49.9%, children : 43.8% - 58.8%) in the studied population is considerably high and is the main determinant as is quoted by many other authors [Chiba et al 1980, Blain et al 1981, Ismail & Rahman 1990].

The findings of this study showed that both *Km* and *Vmax* are not significantly affected by variables such as length of disease years, type of therapy and type of epilepsy. Factors such as age [Bauer et al 1982, Bauer et al 1983] and weight [Bauer et al 1982] which have been found to be poorly correlated to *Km* has also been observed in the present study. The observed linear relationship between *Vmax* with variables such as weight and age in this study is statistically weak although several authors have earlier found the association to be significant [El-Sayed et al 1989, Ismail & Rahman 1990]. Inter-study differences in study design and patient characteristics and high inter-individual variation among ethnic groups could have contributed to the resultant poor correlation.

The effectiveness of the current target range between 10 - 20mg/L for phenytoin was found to be clinically ineffective in all ethnic groups. This observation can be attributed to the high variation in odds ratios to therapeutic response which is directly linked to high inter-individual variation in *Km* and *Vmax* among all ethnic groups. Other factors that might also affect therapeutic response are age, weight and sex. These findings thus indicate that the target range should only be used as a guide and the use of phenytoin in treatment should be individualised.

The possibility of inter-ethnic differences for carbamazepine has been denied by Eichelbaum et al 1985 but the present review of published literatures showed otherwise. Reports by Korean [Yoon et al 1996] and Chinese [Lin et al 1991] authors provide data on the existence of inter-ethnic differences in mean half-lives between the studied subjects and those of Caucasians. Comparison of reported mean clearances between Malaysian and Japanese patients also showed that ethnic differences are likely.

The present study observed a linear relationship between clearance and dose and the transformation of which has made its possible to predict the probable curvilinear relationship between carbamazepine dose and serum concentration. These findings are in line with studies reported by Kudriakova et al (1992) and Summers and Summers (1989) although reports by Hartley et al (1990) and Larkin et al (1991) found otherwise. Evidence of a linear relationship between clearance and dose also indicates the dose-dependent characteristic of carbamazepine. Both these findings have been reported by other researchers [Eadie & Tyrer 1980, Rambeck et al 1987]. This relationship is also found to be affected by age but not with ethnicity.

The lack of efficacy of carbamazepine therapy within the target range of 4 - 12 mg/L can be explained by the high variation in odds ratio for therapeutic response among all ethnic groups. The effect of the high variation in odds ratios can be demonstrated for both adult and paediatric Malay, Chinese and Indian population ranging between 0.39 to 1.00. The higher variation in odd-ratios among adult Indian(0.99) and Chinese(1.00) than that of Malay(0.42) patients is an indication of the genetic diversity in the two former populations. The high degree of variations also lead to the differences in response within the target range between Malay, Chinese and Indian patients being statistically insignificant. The lack of therapeutic differences can be contributed also by the high inter-individual variation in the ratio of serum concentration to dose in the present patients groups.

Report on inter-ethnic differences for valproic acid has yet to be published. The comparison of results from two studies by Chiba et al (1985) and Botha et al (1995) however showed that clearances between Japanese and South African Black and Indian monotherapy treated patients are different. Evidence to compare similar differences on other ethnic groups cannot be ascertained as the ethnic compositions of most published reports were unknown.

The present study showed that the relationship between valproic acid dose and serum concentration is linear in monotherapy patients. These observations also showed that the relationship is affected by associated therapy. Although similar findings have been reported by Tisdale et al (1992) and Mesdjian et al (1984), linear relationship in polytherapy treated patients have also been published [Hassan et al 1976, Rodenburg et al 1977]. These latter studies however failed to provide statistical evidence for their findings.

The influence of age, weight or ethnicity on the observed linear relationship between dose and serum concentration were found to be insignificant. Nevertheless, the observed high inter-individual variation demonstrated by all ethnic groups of the current study is in concordance with the findings obtained by the above studies.

Poor correlation between clearance and dose in this study showed that valproic acid has no dose-dependent properties. This finding is in line with reports by Dodson et al (1981) and Gram et al (1980) where they attributed the observed relationship to valproic acid concentration-dependent protein binding properties. The current results however contradict the conclusions made by Tisdale et al (1992) of a linear relationship which they commented as a consequence to the saturation of binding sites of the concentration-dependent protein binding properties of valproic acid.

Comparing valproic acid efficacy with respect to its target concentration range showed that difference between controlled and uncontrolled Malay, Chinese and Indian patients were statistically insignificant. The influence of ethnicity on the poor correlation in efficacy is also insignificant. These findings can be attributed to the considerably high variation in odds ratios which range between 0.15 - 0.85 for all ethnic groups. The lack of therapeutic differences can be contributed also by the high inter-individual variation in the ratio of serum concentration to dose in the present patients groups. Again these findings indicate that the cautious use of target range is essential in the management of patients with valproic acid.

Reports on ethnic-specific studies of phenobarbitone are insufficient to verify it existence. Review of literature showed that inter-ethnic differences are possible in younger populations where level/dose ratios obtained by Japanese and European authors revealed variation in results for children in the age range of zero to 15 years old.

The studied Malaysian adult population nevertheless showed ethnic differences among Malay, Chinese and Indian patients is highly improbable. It should be mentioned that phenobarbitone is currently not a popular drug of choice due to the availability of newer drugs that are more efficacious with less side-effects.

Relationship between phenobarbitone dose and serum concentration in Malaysian base adult population is found to be linear and the correlation and relationship between clearance and dose is poor and non-linear. These findings are concurrent to earlier reports by Browne et al (1985), Svensmark and Buchthal (1963) and Strandjord and Johannessen

(1977) and supported the theory that phenobarbitone pharmacokinetics are not dose dependent.

The efficacy of phenobarbitone in the target range of 15 - 40mg/L showed there were no differences between controlled and uncontrolled of Malay, Chinese and Indian patients. The high variation in odds ratios displayed by the studied population (range between 0.14 to 0.65) can explain the reason for the absence of inter-ethnic difference among Malay, Chinese and Indian patients.The lack of therapeutic differences can be contributed also by the high inter-individual variation in the ratio of clearance to dose in the present patients groups.

Finally, since serum concentration monitoring can be an important tool in identifying and resolving actual and potential problems during therapy, the need to pay special attention to recent studies evaluating the differences in outcomes in specific ethnic groups may be crucial in antiepileptic drug therapy of these drugs. Incorporating inappropriate population pharmacokinetics information could lead to misdirect dosage adjustments and would most likely offer little benefit in the provision of clinical care.

Chapter 12

Conclusion

Establishing the influence of ethnicity on the population pharmacokinetics for established antiepileptic drugs is restricted by the limited numbers of published ethnic-based studies and inter-study variations. Review of the literature found that inter-ethnic differences are evident in drugs such as phenytoin and carbamazepine but these differences are still unclear for valproic acid and phenobarbitone. Pharmacokinetic parameters that are associated with phenytoin inter-ethnic differences are the Michealis-Menten constant(*Km*), which is equal to the plasma concentration at which the rate of metabolism is one-half the maximum, maximum metabolic rate(*Vmax*), half-life($t_{1/2}$) and clearance(CL). By contrast, half-life and clearance were the two main pharmacokinetic parameters linked to inter-ethnic differences in carbamazepine, valproic acid and phenobarbitone.

In the present study it was found that routine monitoring of established antiepileptic drugs in relation to their target ranges is clinically unimportant and had little bearing on achieving good therapeutic response. Therapeutic response, which was measured by the degree of seizure control was not dependent on inter-ethnic differences. These results were confirmed as the differences observed between Malays, Chinese and Indians pooled odds ratio of controlled and uncontrolled patients in the defined therapeutic ranges were statistically insignificant. The apparently high inter-individual variation displayed in all ethnic groups showed that the use of these drugs should be individualised. Age was not an important factor for the association between therapeutic response and the target therapeutic range. This was confirmed by the pooled odds ratio of carbamazepine and valproic acid. Similar observations were not conclusive for phenytoin and phenobarbitone due to inadequacy of data. These observations are thus in-line with the recommendation outlined by the Commission on Antiepileptic drugs, International League Against Epilepsy (1993).

The relationship between dose and serum level for carbamazepine is found to be curvilinear and thus verified its dose-dependent properties. Linear relationship between valproic acid dose and serum level is highly variable and found to be highly

significant in patients on valproic acid monotherapy. Phenobarbitone dose and serum concentration relationship are highly correlated. This study also showed that neither valproic acid nor phenobarbitone displayed any dose-dependent kinetics.

Factors such as weight, age or ethnicity has no influence on the relative relationship between dose and serum concentration for carbamazepine, valproic acid and phenobarbitone. The main factor that could have affected this relationship is the high interindividual variation between patients which clearly indicates that the use of these drugs must be individualised.

The linear relationship between clearance and dose is used in determining the trend and influence of ethnicity on the population pharmacokinetics of carbamazepine. As for valproic acid and phenobarbitone, a similar linear relationship between serum concentration and dose is used to determine for inter-ethnic differences. Evidence of inter-ethnic differences for all three drugs among Malay, Chinese and Indian epileptic population in Malaysia was found to be statistically insignificant. High coefficient of variation of the ratio of clearances to dose(carbamazepine) and serum level to dose(valproic acid and phenobarbitone) displayed in all ethnic groups showed that interindividual variation is the important factor that could affect the observed relationships.

Evidence showed that there are inter-ethnic differences in phenytoin pharmacokinetic parameters (*Km,mg/L* and *Vmax,mg/day*) among Malay, Chinese and Indian adults patients of Malaysia but not for paediatric patients. Elimination rate constant with a value equal to the plasma concentration at which the rate is one-half the maximum (*Km*) was found to be lower for Chinese and differed significantly to that of Malay and Indian adult patients. Differences between Malay and Indian adult patients were insignificant. Metabolic rate capacity(*Vmax,mg/kg.day*) of all three ethnic groups were statistically similar.

The relationships between *Km* with age and weight in both adult and paediatric patients were non-linear. Relationship between *Vmax(mg/kg.day)* with age or weight was weak although there is an inclination towards a linear relationship in paediatric patients.

Finally, it should be mentioned that the results of this study are unique in that no other Malaysian study had ever reported or compared the effectiveness of the

current therapeutic range or the differences in dose and serum concentration relationship between Malaysian ethnic groups. This study showed that Malaysian Malays, Chinese and Indians did not differ significantly in handling carbamazepine, valproic acid and phenobarbitone. However, inter-ethnic differences between Malaysian Chinese with both Malay and Indian patients signify that the use of phenytoin on Malaysian patients must be adjusted to their racial background. This thesis support the use of ethnic specific phenytoin pharmacokinetic parameters during therapy.

Chapter 13

Future work

The present study has proposed population parameters for phenytoin, carbamazepine and phenobarbitone that are derived for the Malaysian population. These parameters are of clinical importance since these can simplify the estimation of serum concentration of these drugs during chronic therapy. However, the values of these population parameters need to be evaluated and it is suggested that a prospective study, that utilises the above recommendations on Malaysian epileptic patients need to be initiated.

The proposed study should include all three important factors that can affect the overall outcome. First, blood or serum monitoring must be fixed for time of sampling which can either be the pre(trough) level or consistently done on the same time at every monitoring. Secondly, changes in medication due to other illnesses or fasting in the month Ramadan for Moslems [Aslam et al 1997] can jeopardised treatment startegy and should either be included as another variable in the analysis or excluded during the selection process. Thirdly, paediatric patient's should be categorically group in smaller age range, such as lower or higher than 12 years old due to the changes in the liver metabolic capacity. Similarly, geriatric patients(age more than 65 years) should be grouped separately.

The results obtained for phenytoin, carbamazepine, valproic acid and phenobarbitone are nevertheless lacking in statistical sophistication. Since the findings showed that high inter-individual variation can be the crucial factor for the variable degree of therapeutic response, the present method of estimating inter-individual variation does not permit for the 95% confidence interval to be calculated. This thus hindered any possibility to test for differences in inter-individual variation among the three ethnic groups. Inter-ethnic differences in inter-individual variation can be calculated by using specialised computer software such as NONMEM (Non-Linear Mixed Effect Model) where estimates of both intra-individual and inter-individual variation can be determined. Thus, it is proposed that the current data be reanalysed based on the NONMEM software where it is hoped that results from the current finding can be further validated.

Part 4

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Part 5

Appendices

Hospitals visited

	Hospitals	Date
1.	Hospital Sultanah Aminah, Johor Bahru, Johor.	3-10-96 to 24-10-96
2.	Hospital Permai, Tampoi, Johor Bahru, Johor	26-10-96 to 14-11-96
3.	Hospital Tengku Ampuan Rahimah, Kelang, Selangor.	16-10-96 to 7-11-96
4.	Hospital Ipoh, Ipoh, Perak.	9-12-96 to 23-12-96
5.	Hospital Universiti, Kuala Lumpur.	10-11-96 to 13-11-96
6.	Hospital Pulau Pinang, Pulau Pinang.	15-11-96 to 7-11-96

mmm/9.8.96

Appendix 5-2

Date :

Content of worksheet:

1. **Name of Hospital:**

2. **Hospital bed size:**

3. **Number of Specialist(Neurologist,Psychiatrist):**

4. **Type of facility:**
   - -1. outpatient clinic
   - -2. Specialist clinic
     - a.Neurology
     - b.Psychiatric
     - c.Paediatric
     - d.Medical
     - G.Others. Please specify:
   - -3. inpatient(hospitalized)
     - a.Neurology
     - b.Psychiatric
     - c.Paediatric
     - d.Medical
     - e.Intensive care
     - f.Geriatric
     - g.Others. Please specify:

5. **Patient Biodata:**

a.	Registration number	:	
b.	Age	:	(years)
c.	Sex	:	(M/F)
d.	Weight	:	(kilogram)
e.	Height	:	(cm)
f.	Race Malay-M Chinese-C Indian-I Other races-O	:(M/C/I/O)	
g.	Patient doctor Houseman Medical	:(HMO/MO/SP)	

Officer-HMO
Medical Officer-MO
Specialist-SP

h. Diagnosis :i.
ii.
iii.
iv.
v.

i. Epilepsy Classn : (Partial,focal-P)
(Generalized-G)
(Unclassified-U)
(Special syndrome-S)

j. Seizure type : (Partial-F)
(Generalized-L)
(Unclassified-D)
(Unknown-K)

k. Length of disease : (years)

l. Cocomittant disease :

m. Drug therapy :i.
ii.
iii.
iv.
v.
vi.
vii.

n. Length of therapy :i.
ii.
iii.
iv.
v.
vi.
vii.

o. compliance :poor/good

o. Present condition
a. Seizure control: (Percentage)
b. others diseases:(control/uncontrol)

6. Laboratory Results

a. Serum levels(ug/ml)-Total
Date taken :1. 2.
Time taken :1. 2.
Type of assay :(FPIA-f/HPLC-h/EMIT-e)
Done by :(Biochemist-b/Pharmacist-p)

	i.	Carbamazepine	:1.	2.	
	ii.	Phenytoin	:1.	2.	
	iii.	Phenobarbitone	:1.	2.	
	iv.	Sodium valproate	:1.	2.	
b.	Liver function Test				
	Date		:		
	SGOT		:N/H/L		
	APT		:N/H/L		
	Bilirubin		:N/H/L		
	Albumin		:N/H/L		
	Globulin		:N/H/L		
c.	Renal function test				
	Date		:		
	Urea		:N/H/L		
	Sodium		:N/H/L		
	Potassium		:N/H/L		
	Chloride		:N/H/L		
	Calcium		:N/H/L		
	Bicarbonate		:N/H/L		
	Carbonate		:N/H/L		
	Creatinine		:N/H/L		
d.	Blood test				
	Date		:		
	Red Blood Cells		:N/H/L/AB		
	Platelets		:N/H/L/AB		
	WBC		:N/H/L/AB		
	Ph		:N/H/L		
e.	Blood Sugar				
	Date		:		
	Glucose		:N/H/L		
g.	EEG		:N/AB		

(Abbreviation:N-Normal, H-High, L-Low, Abnormal-AB)

7. **Side-effects**

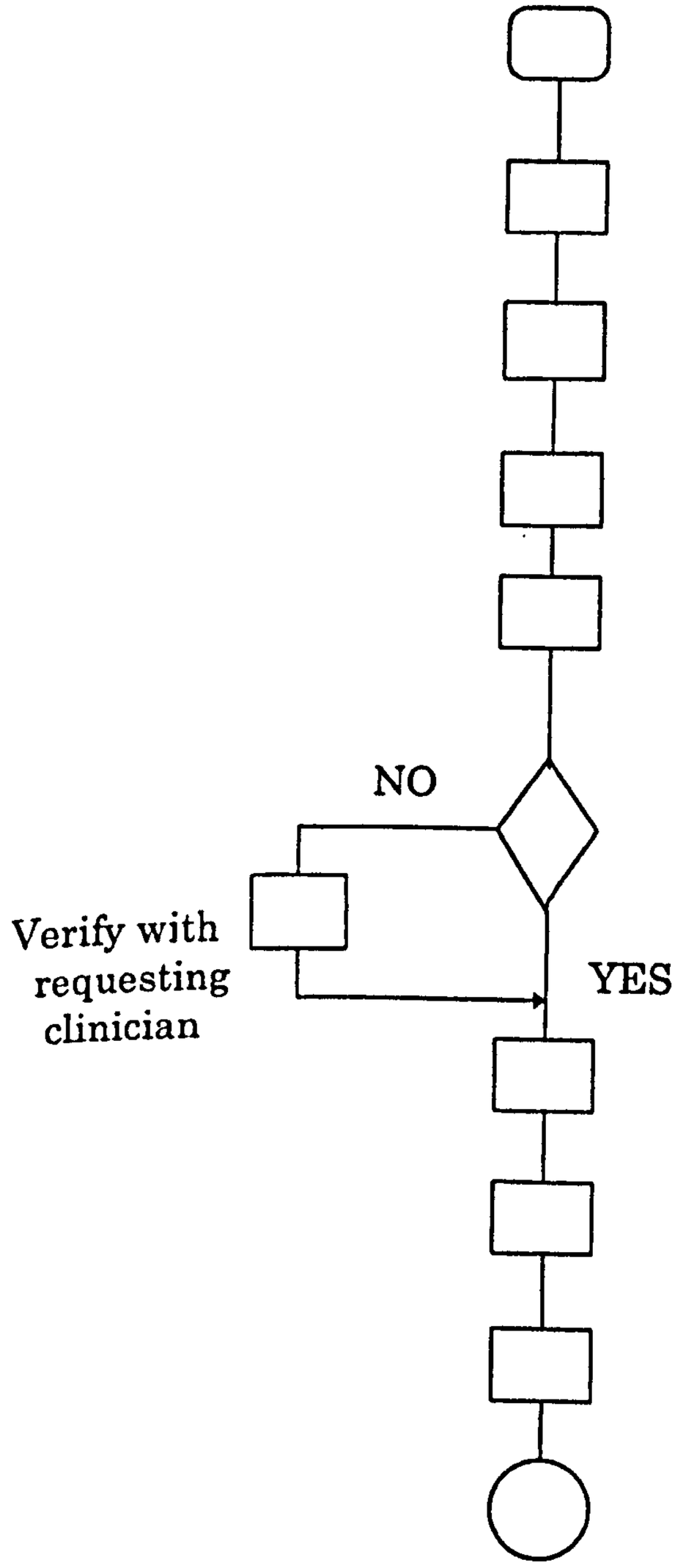
Drowzy-DZ	Dizzy-DI
Headache-HA	Nausea-NU
ataxia-AT	Diplopia-DP
Unsteadiness-US	Behavioral changes-BC
Oculogyric crises-OC	Asterixis-AS
Insomnia-IN	Orofacial Dyskinesia-OD
Hallucination-HU	Taste change-TC
Allergic Rash-AR	Haematological-HE
Hepatic-HP	Hypersensitivity-HS
Steven Johnson Syn-ST	Lyell Syndrom-LS

Dermatitis-DE
Hypervolumia-HV
Edema-ED
Sedation-SE
Depression-DP
Decreased libido-DL
Acne-AC
Lack of coordination-LC
Gum hypertrophy-GH
Red cell aplasia-RC
Folate deficiency-FD
Vit.D deficiency-VD
Hae'hagic pancreatitis-HS

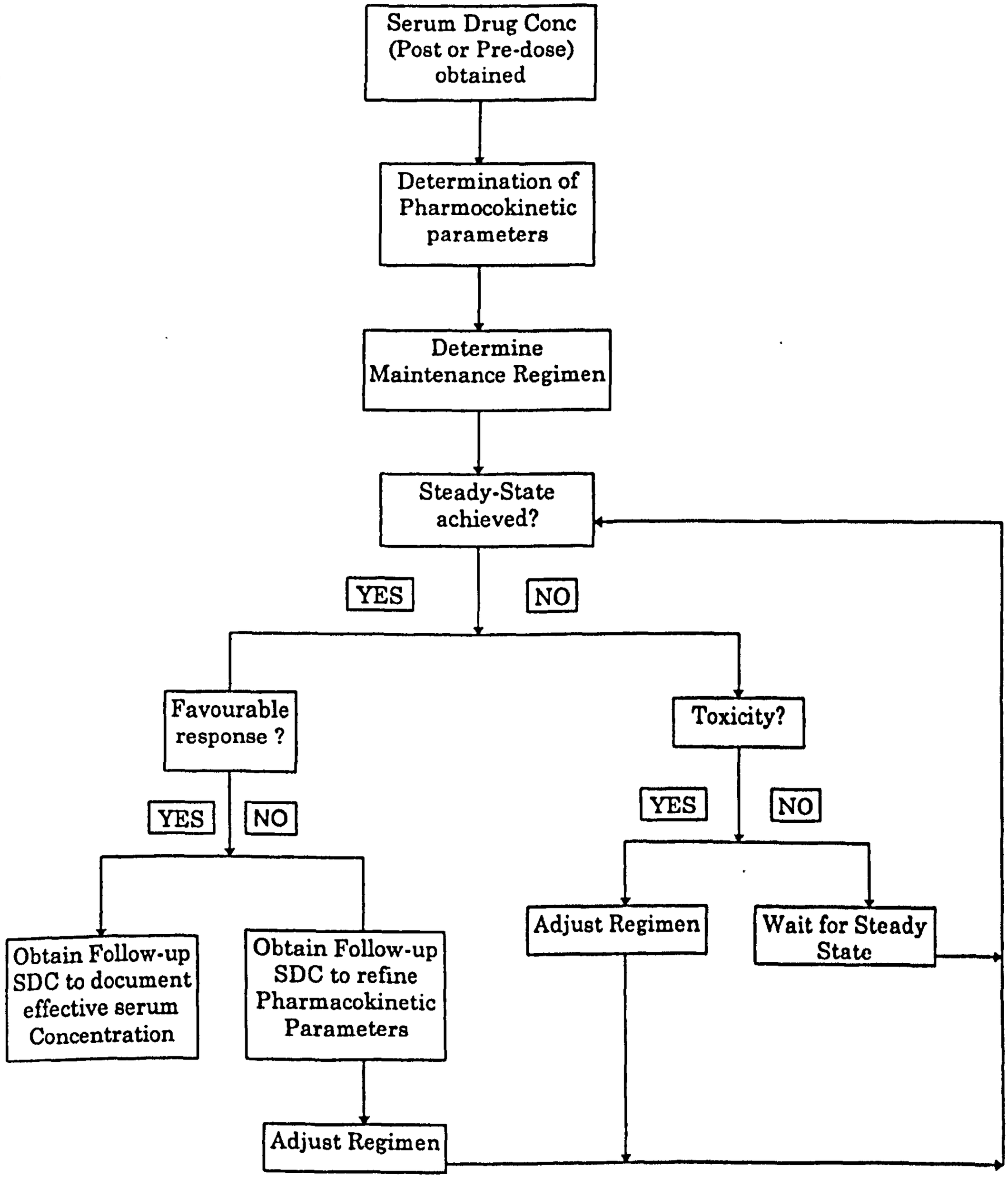
Hyponatremia-HN
Weight Gain-WG
Anorexia-AN
Mental slowness-MS
Hyperactivity-HY
Impotence-IM
Hirsutism-HR
Dystonia-DS
Thrombocytopenia-TH
Agranulocytosis-AG
Megaloblastic anemia-MA
Hyperglycemia-HY

Appendix 5-3a

Flow chart for Therapeutic Drug Monitoring



Serum Drug Concentration Assessment



THERAPEUTIC DRUG MONITORING
SERVICE
HSAJB
2231666 EXT2016

TDM NO:.....
DATE:.....

TO BE FILLED BY REQUESTING DOCTOR

NAME OF DOCTOR:..... SIGNATURE:.....

1. **PATIENT BIODATA**

NAME: ..... R/N(IC):.....
AGE:..... SEX:.....
WT(KG):..... HT(CM):.....
RACE:..... WARD:.....
SMOKER: YES/NO ALCOHOLIC: YES/NO

2. **MEDICAL HISTORY**

CLINICAL DIAGNOSIS:.....
VS:.T.....BP.....HR.....RR.....
CVS(AF/DRNM ETC):.....
DIALYSIS:YES/NO
FITS' CHART:.....
PRESENT CONDITION:.....

3. **RX**

DATE	*DRUG REGIMEN*	*DATE*	*DRUG REGIMEN*

4. **INDICATION FOR REQUEST(PLEASE TICK)**

- ▩ THERAPEUTIC CONFIRMATION
- ▩ COMPLIANCE
- ▩ SUSPECTED TOXICITY
- ▩ LOW THERAPEUTIC RESPONSE

5. **DRUG(S) FOR TDM**

DATE STARTED	*DRUG REGIMEN(ROUTE, DOSE, FREQUENCY,TYPE OF FORMULATION AND BRAND)*	*PRE-SAMPLING TIME*	*DOSING TIME*	*POST-SAMPLING TIME*

6. LAB INDICES

RFT	*LFT*	*ELECTROLYTE*	*HEMATOLOGY*	*OTHERS*
UREA:	*ALB:*	*Na:*	*RBC:*	*RBS:*
SCR:	*GLB:*	*K:*	*WBC:*	*ABG:*
(PRE-RX)	*BIL(T):*	*Cl:*	*%LYM:*	
SCR:	*BIL(U):*	*Ca:*	*PLT:*	
(POST-RX)	*ALP:*	*Mg:*	*PT:*	
	AST:	*Po_4:*		
	ALT:			

7. PAST TDM HISTORY

DATE	*DRUG REGIMEN*	*PRE-LEVEL*	*POST-LEVEL*	*REMARKS*

✂.....

THERAPEUTIC DRUG MONITORING
JABATAN FARMASI
HSAJB
2231666EXT2016

PATIENT NAME:.....R/N:.....WARD:.....

TDM NO:.....DATE:.....

DRUG REGIMEN	*PRE-LEVEL*	*POST-LEVEL*

DESIRED THERAPEUTIC RANGE:.....

.....

COMMENTS: Ke:.....hr^{-1} $T_{1/2}$:.....hr V_d:.....L/KG

.....
PEGAWAI FARMASI Y/M
TDM JABATAN FARMASI
HSA

Appendix 5-4 : Comparison of the relative advantageous and disadvantegeous of the various phenytoin assay methods

Tests	Analytical methods						
	HPLC	GC (on column methylation)	GC (pre-column methylation)	RIA	EMIT	FPIA	SP
Specificity	1	1	1	2	2	2	4
Sensitivity	1	1	1	1	1	1	3
Metabolite analysis	c	c	c	-	-	-	-
Plasma/serum	1	1	1	1	1	1	2
Urine	2	2	2	?	?	?	3
Saliva	2	2	2	?	?	?	-
Minimum sample required(vol)	0.2	0.5	0.5	0.2	0.2	0.2	1.0
Analysis time	2	2	3	2	1	1	3
Reagent cost	2	2	2	4	4	4	1
Tech. cost/assay	3	2	2	2	1	1	2
Equipment cost	3	2	2	4	2	2	1
Training	3	4	4	2	1	1	2

[a] Arbitrary ranking scale of 1(excellent) to 5(poor) with a score of 3 being average or nominal
HPLC-high performance liquid chromatography, GC-Gas chromatography, RIA-radioimmunoassay, EMIT-enzymes multiplied immunoassay, FPIA-fluorescence polarisation immunoassay, SP-spectrophotometry
[c] - hydrolysis and separate assay required

Appendix 5-5 : Comparison of the relative advantageous and disadvantegeous of the various carbamazepine assay methods

Tests	Analytical methods				
	GLC	HPLC	EMIT (manual)	EMIT (automated)	FPIA
Specificity[a]	1	1	2	2	2
Sensitivity (free levels-ug/ml)	0.5	0.5	2.0(0.5)	2.0(0.5)	0.5
Metabolite analysis	y	y	n	n	n
Assayable sample					
-Plasma/serum	y	y	y	y	y
-Free levels	y	y	y	y	y
-Urine	y	y	n	n	n
-Saliva	y	y	y	y	y
Minimum sample required(vol)	0.5ml	0.5ml	50ul	50ul	50ul
Speed					
- 1 - 10 samples(hr)	4	4	1	<1	<1
- 10 - 100 samples	20/day	20/day	50/day	100/day	100/day
Analysis cost[b]					
-Reagent/tech cost	4	4	3	2	2
-Equipment cost	2	3	3	4	5
Training[c]	4	3	2	2	2
Preference[d]					
-small service	5	3	1	2	2
-large service[e]	4	3	2	1	1
-Research	4	1	3	3	3
Comments	CV≤10%	CV <10%	CV <10%	CV<10%	CV<4%

[a] Arbitrary ranking scale of 1(excellent) to 5(poor) with a score of 3 being average or nominal
HPLC-high performance liquid chromatography, GLC-gas liquid chromatography, EMIT-enzymes multiplied immunoassay, FPIA-fluorescence polarisation immunoassay, SP-spectrophotometry
[b] 1 = least expensive, [c] 1 = least training, [d] 1 = preferred method, [e] simultaneous analysis of phenytoin, primidone and phenobarbitone possible

Appendix 5-6 : Comparison of the relative advantageous and disadvantegeous of the various valproic acid assay methods

Tests	Analytical methods					
	GLC (alkylation)	GLC (no derivative)	HPLC	EMIT (manual)	EMIT (automated)	FPIA
Specificity[a]	2	1	1	2	2	2
Sensitivity-ug/ml	0.5	2.5	0.5	0.5	5.0	0.5
Metabolite analysis	y	n	y	n	n	n
Assayable sample						
-Plasma/serum	y	y	y	y	y	y
-Urine	y	y	y	n	n	n
-CSF	y	y	y	y	y	n
Minimum sample required(vol)	0.5ml	1.0ml	0.5ul	50ul	50ul	50ul
Speed						
- 1 - 10 samples(hr)	4	4	4	1	<1	<1
- 10 - 100 samples	20/day	20/day	20/day	50/day	100/day	100/day
Analysis cost[b]						
-Reagent/tech cost	4	4	3	2	2	2
-Equipment cost	2	2	3	1	1	5
Training[c]	4	4	3	2	2	2
Preference[d]						
-small service	3	5	3	1	4	5
-large service[e]	3	5	3	2	1	1
-Research	1	5	1	3	3	3
Comments	CV≤10%	CV <10%	CV <10%	CV<10%	CV<4%	

[a] Arbitrary ranking scale of 1(excellent) to 5(poor) with a score of 3 being average or nominal
HPLC-high performance liquid chromatography, GLC-gas liquid chromatography, EMIT-enzymes multiplied immunoassay, FPIA-fluorescence polarisation immunoassay, SP-spectrophotometry
[b] 1 = least expensive, [c] 1 = least training, [d] 1 = preferred method, [e] simultaneous analysis of phenytoin, primidone and phenobarbitone possible

Appendix 5-7 : Comparison of the relative advantageous and disadvantegeous of the various phenobarbitone assay methods

Tests	Analytical methods				
	GLC	HPLC	EMIT (manual)	EMIT (automated)	FPIA
Specificity[a]	1	1	2	2	2
Sensitivity (free levels-ug/ml)	0.5	0.5	2.0(0.5)	2.0(0.5)	0.5
Metabolite analysis	y	y	n	n	n
Assayable sample					
-Plasma/serum	y	y	y	y	y
-Free levels	y	y	y	y	y
-Urine	y	y	n	n	n
-Saliva	y	y	y	y	y
Minimum sample required(vol)	0.5ml	0.5ml	50ul	50ul	50ul
Speed					
- 1 - 10 samples(hr)	4	4	1	<1	<1
- 10 - 100 samples	20/day	20/day	50/day	100/day	100/day
Analysis cost[b]					
-Reagent/tech cost	4	4	3	2	2
-Equipment cost	2	3	3	4	5
Training[c]	4	3	2	2	2
Preference[d]					
-small service	5	3	1	2	2
-large service[e]	4	3	2	1	1
-Research	4	1	3	3	3
Comments	CV≤10%	CV <10%	CV <10%	CV<10%	CV<4%

[a] Arbitrary ranking scale of 1(excellent) to 5(poor) with a score of 3 being average or nominal
HPLC-high performance liquid chromatography, GLC-gas liquid chromatography, EMIT-enzymes multiplied immunoassay, FPIA-fluorescence polarisation immunoassay, SP-spectrophotometry
[b] 1 = least expensive, [c] 1 = least training, [d] 1 = preferred method, [e] simultaneous analysis of phenytoin, primidone and phenobarbitone possible

Appendix 5-9

PTNO	HCODE	DCODE	RACE	SEX	AGE	WEIGHT	E-YEARS	SINDEX	THERAPY	PT	CBZ	VPA	PB	TOTAL
1	1	1	1	1	19.00	49.00	5.00	0.06667	0	1	0	0	0	1
8	1	1	1	0	19.00	43.00	12.00	0.10000	0	0	0	1	0	1
16	1	1	1	1	48.00	66.00	12.00	0.00000	0	1	0	0	0	1
26	1	1	1	1	22.00	60.00	0.50	0.00000	0	1	0	0	0	1
36	1	1	1	0	40.00	84.00	25.00	0.00000	0	1	0	0	0	1
37	1	1	1	0	30.00	77.00	22.00	0.00556	0	0	0	1	0	1
48	1	1	1	1	7.00	17.00	3.00	0.00000	0	0	0	0	1	1
54	1	1	1	0	10.00	30.00	5.00	0.01667	0	0	1	0	0	1
62	1	1	1	0	5.00	13.00	2.00	2.00000	0	0	0	1	0	1
64	1	1	1	1	7.00	24.00	3.00	0.00000	0	0	0	0	1	1
65	1	1	1	1	11.00	29.00	6.00	2.00000	0	0	0	0	1	1
72	1	1	1	1	7.00	17.00	4.00	0.28570	0	0	0	1	0	1
76	1	1	1	1	11.00	30.00	9.00	3.00000	0	0	0	1	0	1
93	1	1	1	0	18.00	58.00	6.00	0.00000	0	0	0	1	0	1
98	1	1	1	0	17.00	53.00	8.00	0.71429	0	1	0	0	0	1
109	1	1	1	1	21.00	55.00	10.00	0.01644	0	1	0	0	0	1
110	1	1	1	1	19.00	61.00	12.00	0.01250	0	1	0	0	0	1
111	1	1	1	0	38.00	70.00	4.00	0.05556	0	1	0	0	0	1

Abbreviation: PTNO = patient number, Hcode = hospital code, Dcode = disease code, RACE = 1(Malay),2(Chinese),3(Indian), SEX = 0(Female), 1(male), E-YEARS= number of years that patients has been diagnosed with epilepsy, SINDEX = number of seizures divided by period of observations, THERAPY = 0(monotherapy),1(polytherapy), PT = Phenytoin, CBZ = carbamazepine, VPA = valproic acid, PB = phenobarbitone, TOTAL = total number of drugs prescribed

Ramdomisation strategy for selection a single dose/serum pair

Strategy	Dose/serum level pair available							
	2	3	4	5	6	7	8	9
1st patient	1 ←	2	3	2	1	6	2	2
2nd patient	2 ┘	3	4	3	2	7	3	3
3rd patient		1	1	4	3	1	4	4
4th patient			2	5	4	2	5	5
5th patient				1	5	3	6	6
6th patient					6	4	7	7
7th patient						5	8	8
8th patient							1	9
9th patient								1

Brief description of table;
For example, column with number 3 signify patients with 3 dose/serum level pairs. Thus, for the first patient with this number of dose/serum level pair will have the 2nd dose/serum level pair to be selected. Second patient will then have the 3rd pair selected. Third patient will have the dose/serum level first pair. The cycle will be repeated at the end of dose/serum pair, i.e , with the fourth, fifth and sixth patients with 3 dose/serum level pair will follow the same regime that is described.

Appendix 5-11

Phenytoin :Randomisation strategy to select two dose/serum levels pairs

Selection strategy	Dose serum level pairs available 3	4	5
-1st patient	2,1	4,1	5,3
-2nd patient	3,2	3,2	4,2
-3rd patient	1,3	1,2	3,1
-4th patient		2,4	2,5
-5th patient		3,1	1,2
-6th patient		4,3	2,3
-7th patient			3.4
-8th patient			4,5
-9th patient			5,1
-10th patient			4,1

Brief description of the above table:
Column with the number 3 indicates patients with 3 dose/serum level pairs. The first patient with 3 dose/serum level pairs with follow the number 2,1. Number 2 and 1 signifies dose/serum level of 2nd and 1st to be selected. Second patient with 3 dose/serum level pairs, will have the 3th and 2nd dose/serum level pair(3,2) selected while the 3rd and 1st dose/serum level pairs will be selected for the third patient. The cycle will be repeated at the end of each assignment of each dose/serum level group, i.e, the fourth patient will follow the the number 2,1, fifth 3,2 and six 3,1.

a. Regression Analysis of clearance(liter/kg) and dose(per kilogram body weight)

The regression equation is
Clearance(cl/kg) = 0.0241 + 0.00557 Dose(d/kg)

Predictor	Coef	Stdev	t-ratio	p
Constant	0.024107	0.005546	4.35	0.000
d/kg	0.0055663	0.0002983	18.66	0.000

s = 0.03579 R-sq = 71.8% R-sq(adj) = 71.6%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.44606	0.44606	348.19	0.000
Error	137	0.17551	0.00128		
Total	138	0.62158			

Unusual Observations

Obs.	d/kg	cl/kg	Fit	Stdev.Fit	Residual	St.Resid
91	22.2	0.22584	0.14780	0.00363	0.07803	2.19R
93	25.0	0.23728	0.16326	0.00414	0.07402	2.08R
94	25.7	0.33274	0.16724	0.00429	0.16550	4.66R
95	27.9	0.08931	0.17945	0.00477	-0.09014	-2.54R
98	39.1	0.22932	0.24192	0.00766	-0.01260	-0.36 X
99	42.9	0.20109	0.26266	0.00869	-0.06157	-1.77 X
100	51.3	0.28758	0.30956	0.01108	-0.02197	-0.65 X
101	75.0	0.50322	0.44158	0.01799	0.06164	1.99 X
109	10.0	0.17883	0.07977	0.00346	0.09906	2.78R
119	22.9	0.22949	0.15134	0.00374	0.07815	2.20R
125	40.0	0.31626	0.24676	0.00790	0.06950	1.99 X
129	8.8	0.15645	0.07322	0.00364	0.08322	2.34R

R denotes an obs. with a large st. resid.
X denotes an obs. whose X value gives it large influence.

MTB > Regress 'cl/kg' 2 'd/kg' 'WEIGHT';
SUBC> Constant.

b. Regression Analysis of clearance(L/kg) ,dose(per kilogram body weight and weight)

The regression equation is
cl/kg = 0.0557 + 0.00520 d/kg -0.000765 WEIGHT

Predictor	Coef	Stdev	t-ratio	p
Constant	0.05568	0.01132	4.92	0.000
d/kg	0.0052042	0.0003107	16.75	0.000
WEIGHT	-0.0007650	0.0002415	-3.17	0.002

s = 0.03467 R-sq = 73.7% R-sq(adj) = 73.3%

Analysis of Variance

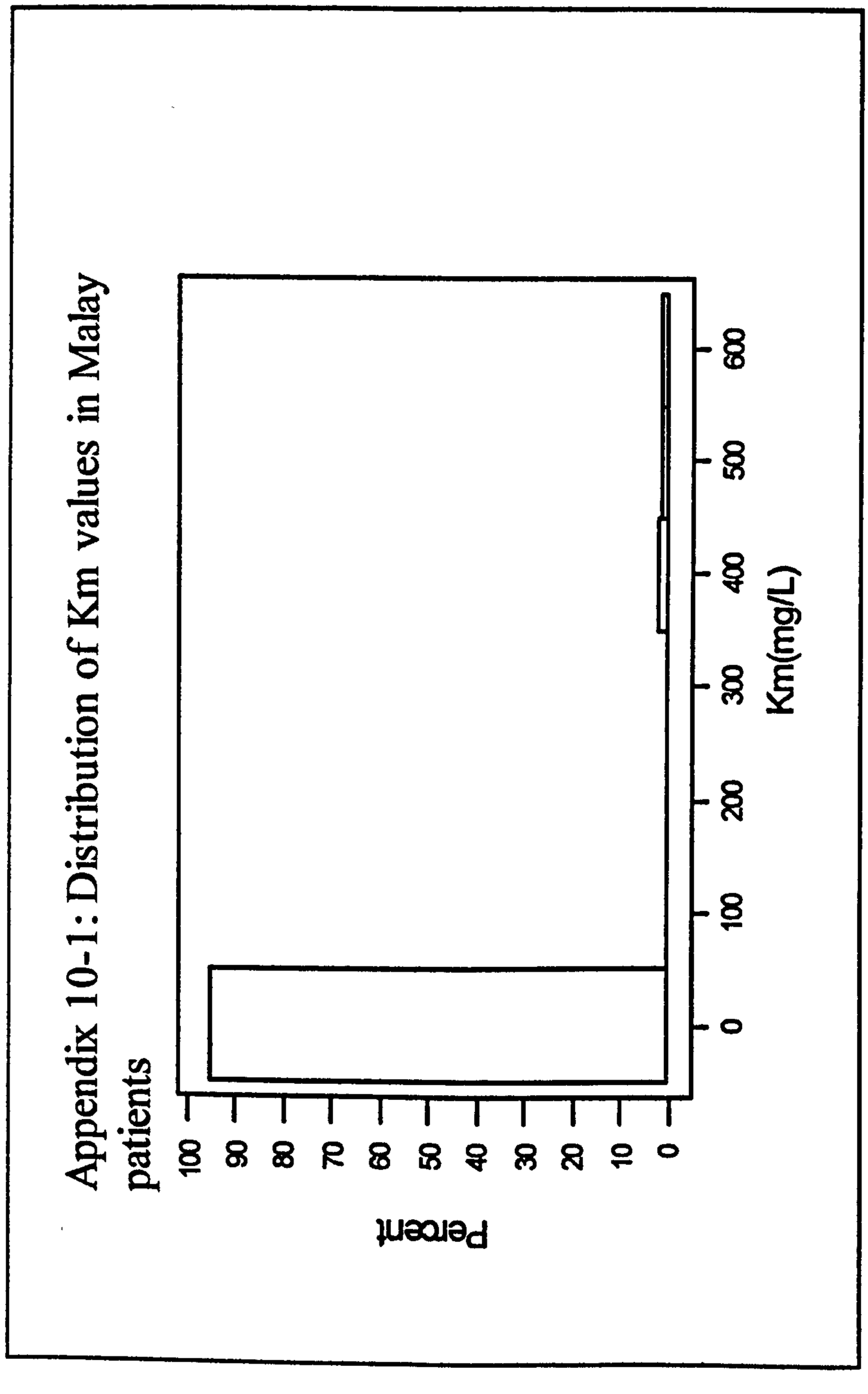
SOURCE	DF	SS	MS	F	p
Regression	2	0.45812	0.22906	190.59	0.000
Error	136	0.16345	0.00120		
Total	138	0.62158			

SOURCE	DF	SEQ SS
d/kg	1	0.44606
WEIGHT	1	0.01206

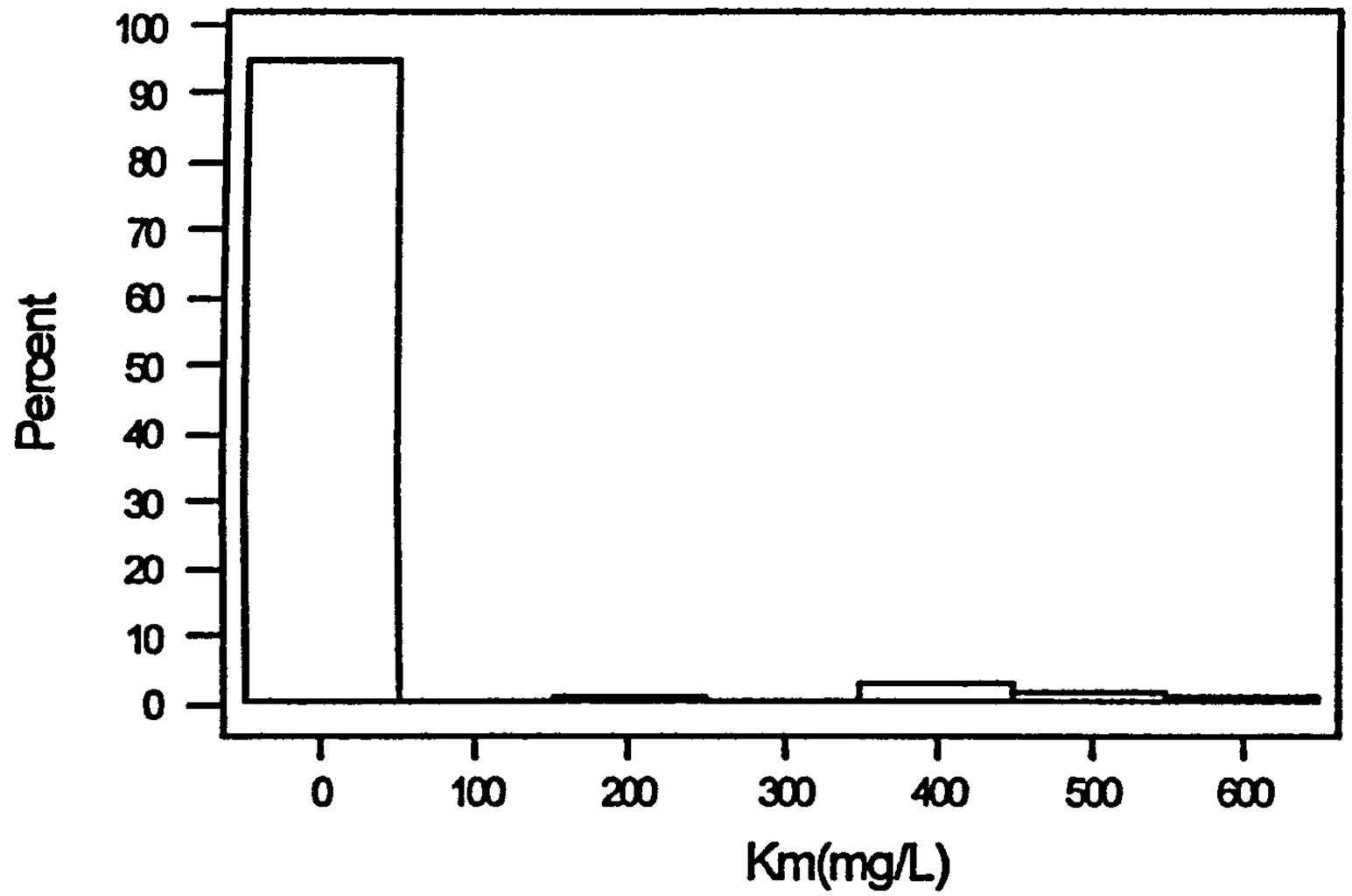
Unusual Observations

Obs.	d/kg	cl/kg	Fit	Stdev.Fit	Residual	St.Resid
34	5.5	0.04996	0.02835	0.00927	0.02161	0.65 X
35	5.6	0.05739	0.03069	0.00884	0.02671	0.80 X
39	10.0	0.08300	0.04652	0.01102	0.03648	1.11 X
94	25.7	0.33274	0.16273	0.00439	0.17001	4.94R
95	27.9	0.08931	0.16802	0.00586	-0.07871	-2.30R
100	51.3	0.28758	0.31361	0.01081	-0.02603	-0.79 X
101	75.0	0.50322	0.43682	0.01749	0.06640	2.22RX
109	10.0	0.17883	0.08477	0.00370	0.09405	2.73R
119	22.9	0.22949	0.14786	0.00378	0.08163	2.37R

R denotes an obs. with a large st. resid.
X denotes an obs. whose X value gives it large influence.


Appendix 10-1: Distribution of Km values in Malay patients
Percent
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
Km(mg/L)

Appendix 10-2: Distribution of Km values in Chinese patients



Appendix 10-3: Distribution of Km values in Indian patients