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POPULATION PHARMACOKINETICS OF ORAL CLOPIDOGREL IN SOUTH INDIAN CARDIOVASCULAR PATIENTS USING NONMEM

Mahender Vattipalli[1], Devender Kodati and Narsimha Reddy Yellu*[1]

Department of Pharmacology and Clinical Pharmacy, University College of Pharmaceutical Sciences, Kakatiya University, Warangal – 506 009, Telangana State, INDIA.

***Corresponding Author Email: ynrku@yahoo.co.in**

ABSTRACT

Objective: *The objective of the study was to perform a Non linear mixed-effects analysis of the pharmacokinetics of clopidogrel, to study the effect of covariates like age, body surface area and creatinine clearance on the population pharmacokinetics of clopidogrel in South Indian Cardiac patients.*

Methods: *A simple, rapid and sensitive isocratic HPLC-UV method for detection and quantification of clopidogrel in plasma had been developed. Intra-and inter-assay variations were <1and <2% respectively. Recovery of clopidogrel was 98-99%. Total 281 blood samples for clopidogrel plasma concentration measurements following a single 75 mg and150 mg /day dose of clopidogrel were obtained from 75 subjects having age in between 18-70 years. The population PK model was built using NONMEM 7.2. The FO and FOCE methods were used to estimate base and covariate models for clopidogrel.* ***Results:*** *One-compartment model with first-order absorption and elimination (ADVAN 2 TRANS 2) was best fit to the plasma concentration-time data of clopidogrel. A combined error model was best described the pattern of residual and between subject variability. The final model estimates of CL and V estimated by FOCE method were 7.6 L/h and 12.6 L.* ***Discussion:*** *There were no past reports on PopPK of clopidogrel in India. With covariate models, no significant decrease was observed in OFV, intra and intersubject variability when compared to the base model. The model that best describe the data following the FOCE method was: Clearance (CL) = ϑ1*EXP (ETA (1)) and Volume (V) = ϑ2*EXP (ETA (2)). No covariates were found as informative for clopidogrel.* ***Conclusion:*** *The POPPK model for clopidogrel has been developed, No covariate has been found to be a factor that affects the individual variability in pharmacokinetics of clopidogrel.*

KEY WORDS

NONMEM, Body Surface Area, Covariate, Residual variability, Clopidogrel.

INTRODUCTION

Clopidogrel inhibits platelet aggregation irreversibly by blocking platelet P2Y12receptors. The absorption of clopidogrel is >50% and is rapid after oral administration. The bioavailability of clopidogrel was increased by food. Peak serum level of the clopidogrel is obtained within 2-5 hours of oral administration of the drug [1]. The mean terminal plasma elimination half-life of clopidogrel ranges from approximately 8 hours following single or multiple doses of clopidogrel given orally. Clopidogrel Cmax and AUC values may prolonged in patients with hepatic and renal impairment [3]. Population pharmacokinetics (PPK) is the study of the variability, its source and its magnitude in populations [4]. This information is used to design dosage regimens that account for individual patient characteristics [5]. Population pharmacokinetics therefore seeks to identify and

measure factors, and define the extent of their influence on the dose concentration interaction. [6]

The knowledge of Population pharmacokinetic is essential in mapping the response surface of the drug of interest, explaining differences seen in the subgroups, developing the dosing strategies, and designing future studies. Diseased humans frequently have disturbed metabolism, which may alter the drug absorption and disposition when compared to healthy individuals. Flexible dosing may prove to be more appropriate [7]. Determining appropriate drug doses requires estimating the pharmacokinetic parameters (such as clearance and volume of distribution) as they relate to covariates, including the precision of these estimates[5,8]. Therapeutic response to cardiovascular drugs can show large intra and inter individual variability therefore it is necessary for serum/plasma concentrations to be monitored during the drug administration if target serum concentrations are to be achieved. The hypothesis tested in this study was that the population pharmacokinetic modeling approach can be used to evaluate and describe the concentration time data collected in the clopidogrel clinical trials. Using this, precise estimates of the pharmacokinetic parameters and their variability have to be quantifiable and significant covariates would be identified.

Population pharmacokinetic approach offers several advantages over the traditional pharmacokinetic approach. Traditional pharmacokinetic methods are often done in a small homogenous group of individuals with intensive sampling, and involve fitting the model to data obtained from each individual separately. The population approach, on the other hand, allows both sparse and intensive data to be used. The sparse sampling approach has enabled pharmacokinetic studies to be carried out ethically in special populations such as neonates, pediatrics, pregnant women, critically ill patients and elderly.

To date, however there is no report on POP PK of clopidogrel although this drug is widely used as anti atherosclerotic drug in India. In present study we developed a PPK model for clopidogrel by analyzing the pooled data obtained from Indian cardiovascular patients. Since clopidogrel shows large individual variability in pharmacokinetics, it is useful to develop a PPK model by integrating the currently available information for clopidogrel. The final PPK model explains several factors that can cause inter individual variability in PK, and the model is able to describe and predict the plasma concentration-time profile for the patients with various backgrounds.

METHODS

Patients and Study design

The population data base consisted of 281 clopidogrel concentrations obtained from 75 (25 female and 47 male) south Indian cardiovascular patients who were on long term treatment with oral clopidogrel tablets. The study design followed was a sparse and random sampling design. The patient group was identified from the patients who visited the cardiology ward of M.G.M. Hospital (Warangal, India) and other private hospitals in Warangal and Hyderabad, India. Informed consent was taken from the patients who were willing to participate in study. Institutional Ethical Committee (IEC) approval was taken before starting the study. Demographic data of all the patients were collected which includes name, age, sex, weight, height, disease status, concomitant diseases (C.V.S, C.N.S., and Renal diseases), and concomitant medications taken along with clopidogrel.

Selection of Patients:

Inclusion Criteria:

Patients of cardiovascular disease, who are treating with clopidogrel.

Patients who are above 18 years, either sex. [9]

Exclusion Criteria:

Severe disability/ malnutrition

Pregnancy & lactation

Age less than 18 years

Any other reasons as decided by physician.

- Human Ethical Committee Registration Number: **UCPSc/KU/BA/04/2013**

Assay of clopidogrel concentrations

Plasma concentrations of clopidogrel were determined by a validated reverse phase high-performance liquid chromatographic method using UV detection and liquid-liquid extraction technique. All plasma samples collected were analyzed by the same procedure at the Department of Drug Metabolism and Pharmacokinetics, University College of Pharmaceutical Sciences, Kakatiya University, India. The chromatographic apparatus was a Shimadzu liquid chromatography system equipped with a LT 10AT VP pump, a SPD 10A VP variable wavelength UV visible spectrophotometric detector and a Rheodyne 20 micro liter loop injector system was used (Shimadzu, Kyoto, Japan). An INERTSIL ODS-3V C-18, 4.6x250mm [Merck Ltd, Mumbai, India] chromatography column was used for analysis.

The mobile phase consisted of Acetonitrile and 0.1% glacial acetic acid with the ratio of 80:20 respectively [10]. The flow rate was 1ml/minute and the eluent was monitored spectrophotometrically at 665nm at room temperature. Ritonavir (20µg/ml) was used as internal standard. Sensitivity of the assay was <50ng/ml. Intra-and inter-assay variations were <1and <2% respectively. Recovery of clopidogrel was 98-99%. Using 500µl of plasma sample, standard curves were linear from 0. 05 to 0.5 µg/ml (r^2 =0. 996).

Model development

The PPK modeling was performed using the NONMEM 7.20 (Version 7, Level 2.0. and FORTRAN power station compiler) with its library subroutines ADVAN2 and TRANS2. A one-compartment linear model with first order absorption was used as a best model. The basic PK parameters were oral clearance (CL/F, L/hr), volume of distribution (V/F, L).The first-Order (FO) and First- order conditional estimation (FOCE) was used throughout the analysis. The POP PK analysis consisted of several major steps like base pharmacokinetic model building, covariate model building, and model reduction to obtain the final model. During the process of building a model, a constant coefficient of variation error model described the inter-individual variability best. This data was analyzed using both FO and FOCE methods in ADVAN2 and TRANS2 and the results are displayed separately. Our results indicated that the one compartment model gave a better OFV (Objective function value) as compared to the two compartment model; hence it was used for describing the pharmacokinetics of clopidogrel.

The inter-individual variability for basic pharmacokinetic parameters was modeled by the log normal distribution, as described in equation 1&2

$$CL/F_j = TVCL.\exp(\eta_{jCL/F}) \quad \ldots\ldots\ldots\ldots (1)$$

$$V/F_j = TVV.\exp(\eta_{jV/F}) \quad \ldots\ldots\ldots\ldots\ldots (2)$$

Where $\eta_{jCL/F}$ is a random variable that represents the difference between individual clearance of the j-th individual (CL/F_j) and the population mean value (TVCL).The random variable $\eta_{jCL/F}$ is a normally distributed with an expectation of zero and a variance of $\omega^2_{CL/F}$.

Residual variability was modeled by the log normal distribution as shown in equation 3.

$$C_{ij} = C_{pred,\, ij.exp}(\varepsilon_{ij}) \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Where C_{ij} is the i-th observed plasma concentration of clopidogrel for the j^{th} individual, $C_{pred,ij}$ is concentration predicted by the PPK model, and ε_{ij} is a randomly distributed variable with mean of zero and variance of σ^2. The minimum value of NONMEM 7.2 Objective Function Value was used as a statistic to choose suitable models during the model-building process. Since the difference in OFV between one model and the other approximates a χ^2 distribution with freedom of the number of parameter difference, a difference in Objective Function Value of 3.84 for 1 degree of freedom ($P<0.05$) was considered statistically significant in the model-building process[11].

Covariate model

Initially, the model was developed without including patient-specific covariates (basic model). Starting from a simple one compartment model, a variety of covariates that could influence the pharmacokinetics of clopidogrel were stepwise added to the basic model (addition method) Statistical significance for incorporation of each covariate was judged based upon change in OFV (ΔOFV). Initially, exponential error models were used to describe the inter-individual variability terms and were included on both pharmacokinetic parameters in the model, and the initial residual error model used consisted of two components: an additive and a proportional component. When an appropriate base PK model had been developed, individual pharmacokinetic parameters were generated in NONMEM and their relationship with covariates graphically explored. Covariates that were evaluated included anthropometric variables, including body weight, height, body surface area (BSA), age, gender, CRCL, smoking history and alcohol consumption. Once a full model was developed which incorporated all possible covariates, each covariate was in turn examined removing one by one (deletion method) to confirm the statistical significance using criterion of ΔOFV with 6.84 ($P<0.01$).The continuous covariates showing correlation with the pharmacokinetic parameters were normalized to their corresponding medians and then introduced into the model as shown by equation 4.

$$P_k = \theta k1 \times (Cov/Cov_{median})^{\theta k2} \quad \text{..... (4)}$$

Where P_k is the PK parameter, θk1 is the typical value of the pharmacokinetic parameter in the population, θk2 is the coefficient of the covariate, Cov is value of Covariate, and Cov_{median} is median of the Covariate in the population under investigation. The least significant parameter (smallest change in objective function) was then removed from the model. This entire cycle was repeated in a stepwise fashion until only significant parameters remained in the "Final" NONMEM structural model [12].

RESULTS

Demographic data for the patients participating in the present PPK analysis is summarized in the following table.

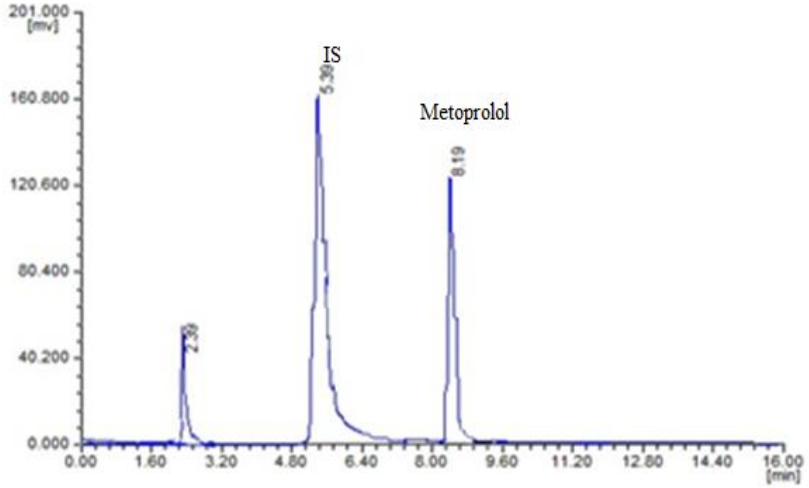
Table 1: Description of the patients participating in the present study

Parameter	Range	Mean (± SD)
Age (years)	28-78	52.78(±5.6)
Body weight (kg)	40– 81	61.2(±5.9)
Dose (mg)	75 – 150	112.5 (±9.3)
Serum level (µg/ml)	0.05– 8.4	1.73 (±0.1)
Sampling time (h)	0.5-12	3.31(±0.6)

All the patients in the study were confirmed to be compliant in taking drugs. The clinician fixed the dosage regimen. After the drug concentration levels reach the steady state, at least 3 - 7 blood samples (4-5ml) from each patient during the clopidogrel treatment, at 0.5, 1, 1.5, 2, 2.5, 4,4.5, 6,6.5, 8,8.5,10,10.5,11,11.5 and 12h before the next dose. These sampling schedules were randomly allocated. Sampling times are not fixed for all patients. Blood samples were collected in EDTA coated tubes and immediately centrifuged at 3000×g for 8 min at room temperature. The collected samples were stored at −80°C until further analysis was carried out.

Clopidogrel Estimation: The mobile phase consists of Acetonitrile : 0.1% Glacial acetic acid in Water(80:20 % v/v). The mobile phase was filtered through 0.20 µm membrane filter. Flow rate was 1 ml/min and effluent monitoring was done at 227 nm. The total run time of the method was set at 10 min. Ritonavir (200 ng/ml) was used as internal standard. Sensitivity of the assay was <50ng/ml. Intra-and inter-assay variations were <1and <2% respectively. Recovery of clopidogrel was 98-99%. Using 500µl of plasma sample, standard curves were linear from 0. 05 to 0.5 µg/ml (r^2 =0. 995).

Fig. 1: HPLC Chromatogram of Clopidogrel and internal standard in human blank serum



Model development: A one-compartment open model with first order absorption was used as a basic structural model, and random variables for inter-individual variability and covariates were added stepwise to develop the PPK model for clopidogrel.

Initially, the model was developed without including patient-specific covariates (basic model). Starting from a simple one compartment model, a variety of covariates that could influence the pharmacokinetics of clopidogrel were stepwise added to the basic model (addition method) Statistical significance for incorporation of each covariate was judged based upon change in OFV (ΔOFV).

In the preliminary screening phase covariates like Creatinine clearance, Age, Comedication, Weight, Sex and BSA does not reduces the objective function. In the forward stepwise model-building the cumulative inclusion of age, creatinine clearance, BSA etc also does not reduces the objective function. No covariate significantly varied the Clearance and volume of distribution. But the inclusion of any covariate in the clearance and volume of distribution has

does not affect the objective function significantly.

Fig.2: Plasma concentration (DV) - time profiles of Clopidogrel in 75 patients.

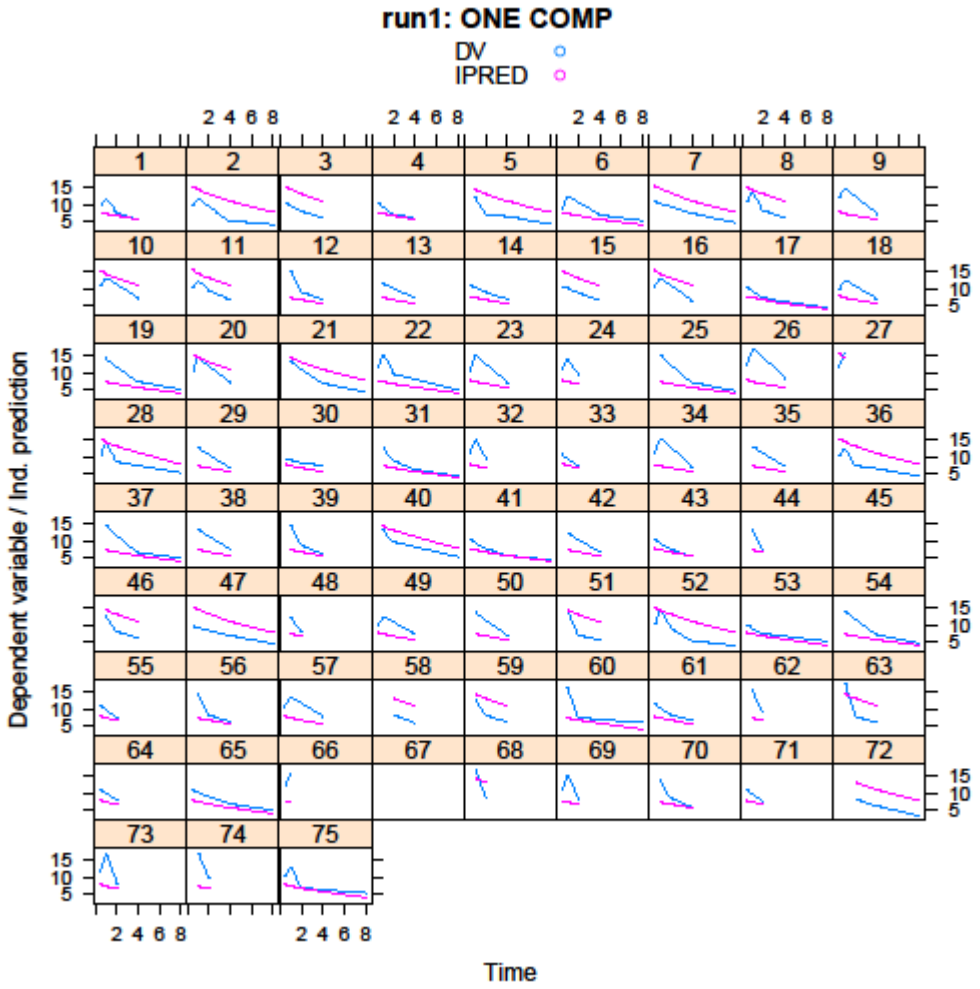


Table 2: Mean pharmacokinetic parameters and inter-individual variability for clopidogrel

Model	OFV	Population estimate (%SE)	Between subject variability (% SE)
Base model/ Final model CL= θ1*EXP (η1) V = θ2*EXP ((η2)	536		
CL (L/hr)		7.6 (22.6)	45% (22)
V (L)		12.6 (27.0)	13% (44)
Residual variability			
Additive error	0.2µg/mL (43.3)		

The final structural model was:

FO Method:

CL = THETA (1) *EXP (ETA (1))

V = THETA (2) *EXP (ETA (2))

FOCE Method:

CL = THETA (1) *EXP (ETA (1))

V = THETA (2) *EXP (ETA (2))

The population pharmacokinetic model parameter estimates obtained by using the final model are given below.

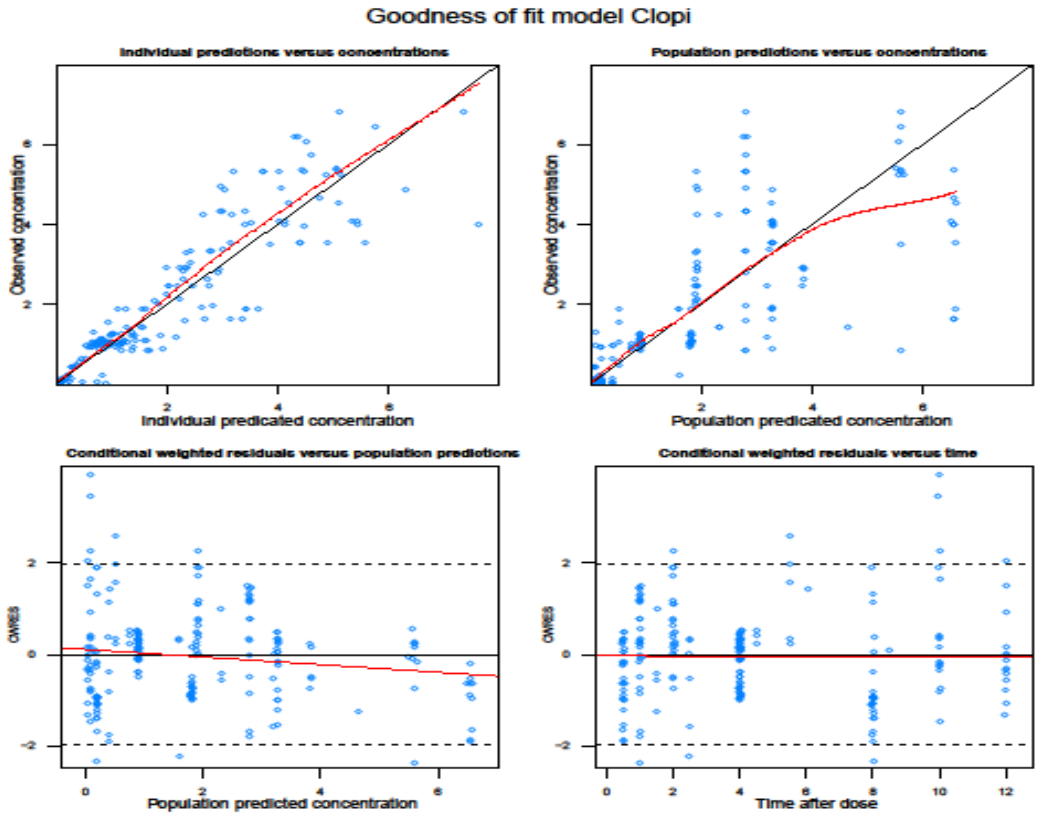
Table 3: Analysis of covariate effect on Clearance of clopidogrel.

HYPOTHESIS	OFV	CHANGE IN OFV
Base Model	-536.542	---
AGE	-535.423	1.119
BSA	-535.502	1.04
SEX	-536.218	0.324
CM	-534.822	1.72
CLCR	-534.425	2.117
SMOK	-534.216	2.326
BSA+CM	-534.218	2.324
BSA+CM+AGE	-535.753	0.789
WT+ AGE+CRCL	-536.328	0.214
BSA+CM+CRCL+AGE	-536.343	0.199

Table 4: Analysis of covariate effect on Volume of distribution of clopidogrel

HYPOTHESIS	OFV	CHANGE IN OFV
Base Model	-536.542	---
AGE	-535.641	0.901
WT+CM	-536.116	0.426
CM	-534.495	2.047
BSA	-534.127	2.415
BSA+CM+AGE	-535.732	0.81
BSA+CM+CRCL	-536.183	0.359
BSA+CM+CRCL+AGE	-535.046	1.496

Fig.3: Goodness of fit graphs for Clopidogrel



DISCUSSION

To date, there are no published population pharmacokinetic models for clopidogrel in cardiac patient population. Our study population is the representative of the Indian cardiovascular patient population. Clopidogrel, it appears that a standard drug produces a large variability in their plasma concentrations. It has been shown that the patients with low volume of distribution and high clearance of these drugs suffering with low efficacy and required more dosage, while the patients with high concentrations are more likely to suffer from adverse events. The principal objective of this study was to account for the inherent individual variability in the population in terms of readily identifiable factors that influence pharmacokinetics of clopidogrel in an Indian cardiac patient population. Estimation of pharmacokinetic parameters in target population is more highly desirable than in healthy volunteers [13].

The PPK model of clopidogrel has been developed based upon the pooled pharmacokinetic data obtained from the cardiovascular patients in India. The CL/F was found to be associated with CRCL but not related to other covariates like age, sex, BSA, gender, smoking and alcohol consumption and the V was related to Age. Our study populations were cardiac in and out patients who were treated with oral clopidogrel. Population values of CL and V for clopidogrel were calculated and final structural models using FO and FOCE method was given.

From these methods it was observed that the CL and the V were found to be not associated with covariates like age, BSA, gender, CRCL, smoking and alcohol consumption. Renal impairment does not show any pharmacokinetic variations in

case of clopidogrel [14]. It will be useful and important to examine the hepatic functional covariates have any effect or not on CL of the clopidogrel. One past study reported that the lower starting doses are recommended for the patients with a history of hepatic impairment, since the number of patients with hepatic impairment was very less in the present data set and this number thought insufficient for the analysis, the effect of hepatic impairment could not be examined in this analysis [15].

In the clinical setting, one compartment model has been usually employed, although several studies reported that the pharmacokinetics of clopidogrel is better characterized by a two-compartment model. In the present study we found that the one-compartment model better describes the pharmacokinetics of clopidogrel by comparing the OFV values obtained after analyzing the data using ADVAN2 TRANS2 which resulted in no significant change in OFV value (Table 3,4). In our study it was observed that the mean population estimates of clearance as 7.6 L/h and volume of distribution (V) as 12.6L.These values were seems to be very low when comparing with a past study conducted in Korean healthy volunteers [13]. No reports were found in the literature regarding the PPK of clopidogrel in any of the other patient population.

The range of clopidogrel concentrations obtained in different patients was 0.05-8.43 (µg/mL), and these values are higher than the values previously reported study conducted in healthy volunteers. The values of the CL/F and V/F are much less when compared with previous literature values obtained from a clinical study conducted in healthy volunteers. This may be due to the differences in the protein binding and differences in the CYP metabolic enzymes of our population with that of other healthy subjects [16].

CONCLUSION

The POPPK model for clopidogrel has been developed based upon the data obtained in the Indian cardiac patient population. No covariate (such as age, sex, BSA, gender, CRCL, smoking and alcohol consumption) has been found to be a factor that affects the individual variability in pharmacokinetics of clopidogrel. The present PPK model well described the individual exposure to clopidogrel and can have a positive impact on management of clopidogrel therapy in the study population.

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***Corresponding Author:**
Prof .Y. Narsimha Reddy
Professor
University College of Pharmaceutical Sciences, Kakatiya University, Warangal - 506009, India.
Work: +91-870-2461-433, Fax: +91-870-245-3508.
Mobile: +919440507384
E-mail: ynrku@yahoo.co.in