International Journal of Drug Delivery 2 (2010) 221-227

http://www.arjournals.org/ijdd.html

Research article

ISSN: 0975-0215

Prospective observational study of vancomycin injection in SLED patient of ethnic Indians

Rakesh Das [*1, 2], Subhabrata Dutta Gupta [1,3], Tapan Kumar Pal [1]

***Corresponding author:**

Mr. Rakesh Das
[1]Jadavpur University, Jadavpur, Kolkata-32, W.B., India
[2]IPS College of pharmacy, Gwalior, M.P., India
[3]Clinical Research Organizations, Novartis, Hyderabad, A.P., India
Tel: +919098896829
Email: rakeshdas_ripsat@yahoo.co.in

Abstract

As the Vancomycin is itself a nephrotoxic antibiotics, so it is sometime recommended to the Slow-low Efficiency Dialysis (SLED) patients against highly resisted infection. In this case, the dose monitoring is strictly maintained after Intravenous injection. The collected blood was analyzed for its concentration in HPLC for 11 patients and the half life was evaluated to study Therapeutic drug monitoring. The $T_{1/2}$ of evaluated vancomycin is 39.12± 6.81 hrs. The mean of the systemic clearance is 16.91±6.99 and mean V_d is 0.57± 0.147. Comparatively the reported study of Mean ± SD of half-life, volume of distribution, and systemic clearance were 43.1 ± 21.6 hours, 0.84 L/kg ± 0.17 L/kg, and 24.3 mL/min ± 8.39 mL/min respectively. Thus the t-test of the means was 0.5828, degree of freedom (df) was 20, standard error of difference was 6.829 and so, the two-tailed P value is 0.5665 i.e. P > 0.5. In ethnic Indian SLED patients, $T_{1/2}$ of mean ± SD of 39.12 ± 6.81 hrs was compared to the Caucasian patients i.e, 43.1 ± 21.6 hrs. And the t-test and P-value is 0.5828 & 0.5665 respectively. Thus it was concluded that the half-life of ethnic Indian patients is less in compare to Caucasians but this difference is not so significant. The half-life of ethnic 8 patients is less than 40 out of 11 patients.

Keywords: Vancomycin assay; Slow-low efficiency dialysis; Pharmacokinetic analysis; Ethnic indians

Introduction

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infection caused by Gram +ve bacteria [1]. Its "last resort" antibiotic after the failure with other antibiotics. Vancomycin prevents incorporation of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG)- peptide subunits into the peptidoglycan matrix; which forms the major structural component of gram +ve cell walls[2]. Its clinically used in the treatment of methicilline resistant *staphylococcus aureus* (MRSA), multiresistant *staphylococcus epidermidis* (MRSE), pseudomembraneous colitis (relapse), prophylaxis for endocarditis and individual serious allergy to penicillins or β- lactum antimicrobials [3]. Vancomycin is intrinsically resistance to gram +ve *Leuconostoc* and *pediococcus* species and gram –ve non-gonococcal Neisseria species, also acquired microbial resistance Enterococci (*Enterococcus faecium* & *E. faccalis*). Vancomycin has common adverse drug reaction with IV include: local pain, which may be severe and /thrombophlebitis, Nephrotoxicity and ototoxicity, also rare adverse effects of anaphylaxis, erythema multiforme, redman

doi:10.5138/ijdd.2010.0975.0215.02032

syndrome, superinfection, thrombocytopenia, neutropenia, leucopenia, tinnitus, dizziness and toxic epidermal necrolysis.

In hospital it was seen that due to above mention gram -ve bacterial infection of resistant species responsible effectively in nephro patients like ARF and CRF, were kept in haemodialysis like Intermediate Haemodialysis (IHD) and Slow- low efficiency Dialysis (SLED) having the last choice of aminoglycoside, vancomycin. SLED is a modified from of intermittend hamodialysis (IHD) with altered blood and low dialysate flow rates[4,5]. SLED has been performed daily over a 6 to 12 hrs period. On focusing its nephrotoxic nature of their antibiotics and its choice of IV administration at last stage of treatment, the requirement exist to control the dose of administration. Regarding this the therapeutic drug dose monitoring is very much essential aspects. So, need arises to identify the SLED patient administered with vancomycin (IV) Clinicians in hospital wards to admitted patients[6, 7]. Such 11 SLED patients were identified in AMRI hospital, Dakuria, Kolkata for 9 month study period. Blood were collected to evaluate the Therapuetic Drug Monitoring purpose with the help sophisticated HPLC instruments. To evaluate pharmacokinetic and Pharmacodynamics parameters, the concentration of the vancomycin was estimated through the peak arises in chromatogram with the help of reverse phase HPLC, isocratic methods. The available pharmacokinetic and pharmacodynamic datas of ethnic patients were estimated and which was comparatively studied with the datas of Caucasian patients.

Materials and methods

Design

We arrange a prospective pharmacokinetic study of **vancomycin** between Sep, 2008 to May, 2009 on 1st class academic level-based superspeciality hospital having 500 beds. Adult patients above 18 years, with actual body weight within 30% of ideal body weight, ethnic Indian, SLED patients received concurrent vancomycin suffering from renal failure.

Patients receiving vancomycin preceeding 5 weeks, with severe liver disease, hospitalized less than 5days; obese, cystic, fibrosis, Malignant and burn patients were excluded from investigation.

For the study of RF patients, the proposal made approved from AMRI Hospital Ethical committee.

Study Protocol

Vancomycin was injected intravenously by the clinicians with doses recommended usually in practice. Patients who started vancomycin after initiation of SLED therapy had serum vancomycin concentration drawn after 1hr of infusion and again drawn befor half an hour of 2nd infusion of vancomycin IV during SLED. The redosing were usually given by clinicians when the serum concentration was less than 20 mcg/ml. The vancomycin concentration was not used for the pharmacokinetic analysis, it was measured to ensure that the vancomycin serum concentration had not fallen too low.

Table 1. Area & Height peaks of standard vancomycin solution.

Conc. Micro.gm	Figures	Retention Time	Height of Peaks [mV]	Area of Peaks [mV.s]
5µgm	FIG-A1	2.57 min	0.012	0.12243
10µgm	FIG-A2	2.57 min	0.021	0.24587
20µgm	FIG-A3	2.57 min	0.031	0.48974
40µgm	FIG-A4	2.57 min	0.049	0.85443
60µgm	FIG-A5	2.57 min	0.051	1.28165
80µgm	FIG-A6	2.57 min	0.10	1.7088
100µgm	FIG-A7	2.57 min	0.148	2.136

Blood was collected when after 1hr of infusion usually in order to know the peak concentration of vancomycin and blood collected before ½ hr of second infusion is to know the trough concentration of vancomycin . The vancomycin peaks concentration were evaluated in HPLC, through BDS C18 (150 X 4.6) under software Clarity lite (Clarity-Chromatography SW) data apex 2003.

Table 2. Patients demography evolved.

Numbers	11
Gender	9/3
Mean Age	60.09 ± 11.98
Mean weight	61.66 ± 11.89
Mean Length	5.59 ± 0.35
Charlson Score	5.26 ± 1.16

SLED Parameter

SLED were performed in the ICU, Nephro-dialysis unit and was performed as a continuous renal replacement therapy over 24 hrs period. SLED therapy was occasionally interrupted because of scheduled equipment cleansing or blood clotting. Blood flow rate (BFR) was set at 200/min with the dialysate running at 100ml/min. Dialysate solution was produced online using concentrated solution and treated tap water. The dialysate solution was drained directly into the sink, of F5, F6, F7 (Fresenies Medical care) hemodialyzers prescribed for SLED.

Table 3. Pharmacokinetic data evaluated in 11 patients after calculation.

Patient's Samples	Plasma Peak conc.	Plasma Tough conc.	Rate of ele -mination (K_{el})	Creatinine Clearance CrCl(ml/min)	Clearance CLs (ml/min)	Vd(L/kg)	T1/2 (hr)	Haemo-dialyser
SP-1	**45**	**16**	0.017	14.45	10.88	0.82	40.76	F6
SP-2	**46.2**	**14**	0.019	44.55	33.46	0.82	36.47	F7
SP-3	**45.6**	**15**	0.018	20.9	15.72	0.51	38.5	F6
SP-4	**44**	**15.6**	0.017	30.36	22.82	0.58	40.76	F5
SP-5	**48**	**13.3**	0.021	17.4	13.1	0.47	33	F5
SP-6	**37.40**	**17**	0.013	27.90	20.97	0.70	53.3	F6
SP-7	**45.87**	**18**	0.015	16.07	12.07	0.46	46.2	F6
SP-8	**46**	**17.2**	0.016	15.17	11.42	0.56	43.31	F6
SP-9	**45.85**	**14.1**	0.019	21.27	20.95	0.45	36.47	F6
SP-10	**45.90**	**12.2**	0.022	16.8	12.69	0.43	31.5	F5
SP-11	**46.27**	**11**	0.023	15.91	11.97	0.45	30.13	F5
Mean ± SD	**45.09 ±2.72**	**14.64 ±2.17**	**NA**	**NA**	**16.91 ± 6.99**	**0.57 ± 0.147**	**39.12± 6.81**	**NA**
SEM							**2.0533**	

Vancomycin Assay

To evaluate the concentration of the vancomycin in the blood plasma analysis, caliberation of the vancomycin estimation parameters were hardly needed with the help of standardization of vancomycin[8]. As the standard vancomycin collected (Central drug lab., Kolkata) and after running into same HPLC machine gives AUC at retention time RT-2.57min . The concentration of phosphate buffer is 50 mmol /L (sonicated) given in isocratic method of 95:5 of HPLC grade water: Acetonitrile. The parameter of the detector is maintained at 270nm wavelength, the flow rate of sample is HPLC cannel is 0.8ml/min, pH maintained of buffer is 3.2 pH. After running the standard solution of the vancomycin through above HPLC machine with above mention parameter the result of peaks obtained is represented in Figure 1.

The above peak obtained in the HPLC was ploted as the voltage (mV.s) Vs time intervals for 5,10,20,40,60,80,100µgm / ml concentration in Figure 1. According to that the Table 1 represents the Height of peak (mV) , Area of peaks(mV.s) , Retention time (RT) against respective concentration. In Table 1, FIG-A1,A2,A3,A4,A5,A6 & A7 represents the peaks plotted, which is remarked in Figure 1.

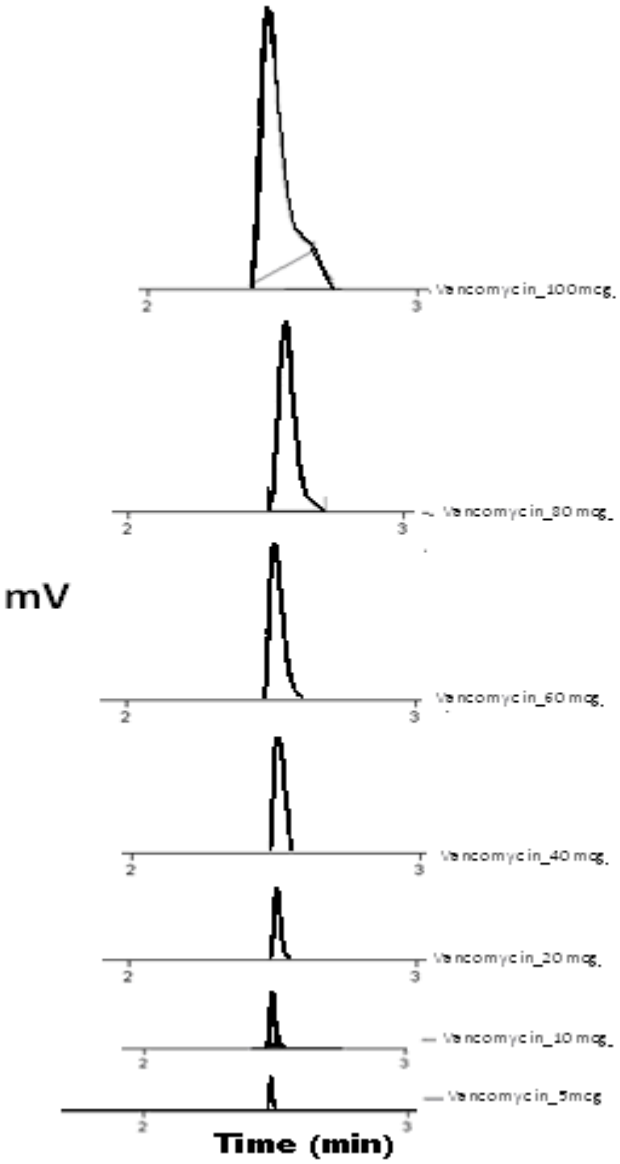

Figure 1. Standardization of vancomycin AUC under following parameters.

According to figure 2, the area of the peaks (AUC) in mV.s were taken to plot a near about straight or linear graph with the respect of concentration (µg/ml). This graph represents the equation: **y=a+b*x;** linear regession: **r^2 = 0.99428;** Slope**: 0.050915;** Intercepts: **-0.0624.** That's why on going through the linear regression, this slop of graph was considered.

Standardization of vancomycin was done after many variation in parameters in order to maintain the maximum retention time so that overlapping of the peaks area with the other drugs in the serum can be avoided.

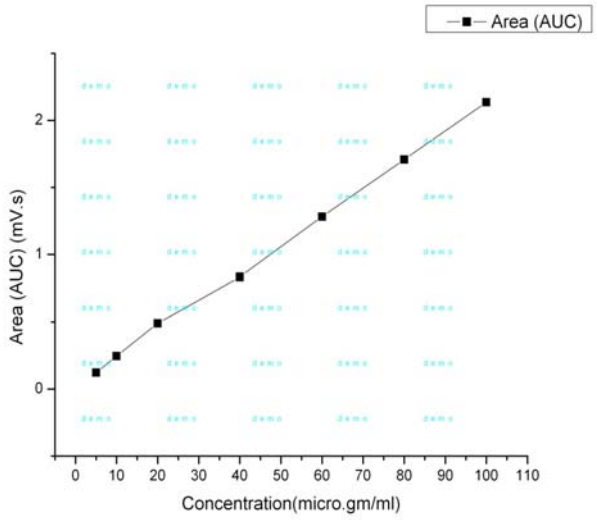

Figure 2. Standardization curve of vancomycin; Area (AUC) of peak Vs Concentration µg/ml.

After standardization of vancomycin concentration with AUC, the concentration for the peak level and trough level of 11 SLED patients were evaluated. So, Blood samples were collected from the patient in a red tube and was transferred to the laboratory for centrifugation at a 5000 rpm serum collected and stored at -20 °C .Before experiment it was mixed with di-methyl-sulfoxide [DMSO] and internal standard I.S., centrifuge at 5000rpm supernatant spike serum was then collected in 20 mcL in injection to run through HPLC. Internal standards**(IS)** are very much essentials for the bioassays. Here, in this HPLC assay five Internal Standard (IS) were used – **Imipramine, Nebivolol, Ondanosatron, Aceclofenac, Ibuprofen** having retention time 2.56, 2.54, 2.49,2.83 2.36 min respectively. From above IS ondanosatron was selected of 60 µg/ml to the injected 11 samples shown in Figure 3 & 4 for peak and tough level on 5 patients.

Concentration of vancomycin (vanco) was estimated from AUC of vancomycin with respect to ondanosatron

(ondan). Thus so evaluate the accuracy in peaks areas of the spiked serum proportionally.

Pharmacokinetic Analysis

Serum Vancomycin concentration were used to determine the elimination rate constant (Kel), elimination rate constant (Kel) , elimination half-life ($T_{1/2}$), volume of distribution (Vd), and systemic clearance (CLs). The study assumed a one-compartment IV bolus model for pharmacokinetics calculation.

The elimination rate constant was determined by performing an unweighted linear least – squares regression analysis with the logarithms of the patients serum vancomycin concentrations. The elimination $T_{1/2}$ was then calculated based on a first-order elimination process, where $T_{1/2} = 0.693/Kel$.

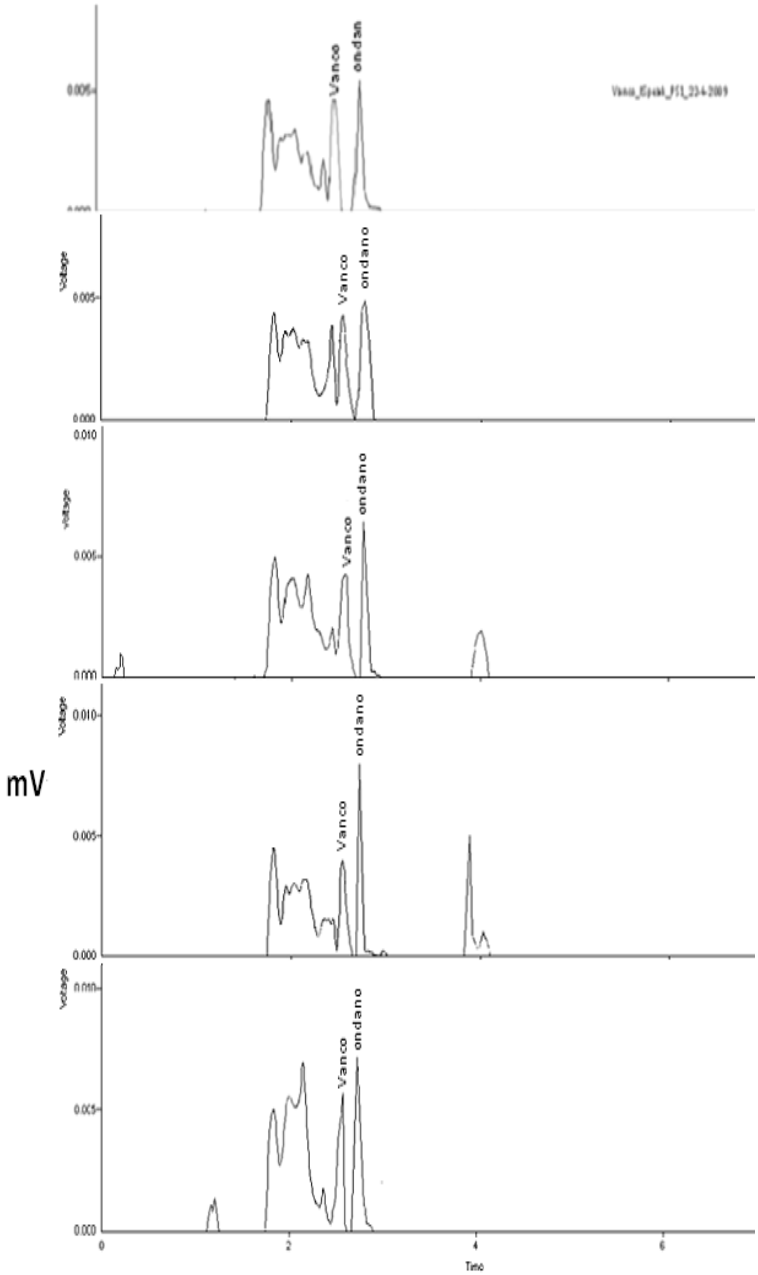

Figure 3. Five patient sample of spiked blood with 60µg/ml IS from SP1 to SP 5 for peak level AUC.

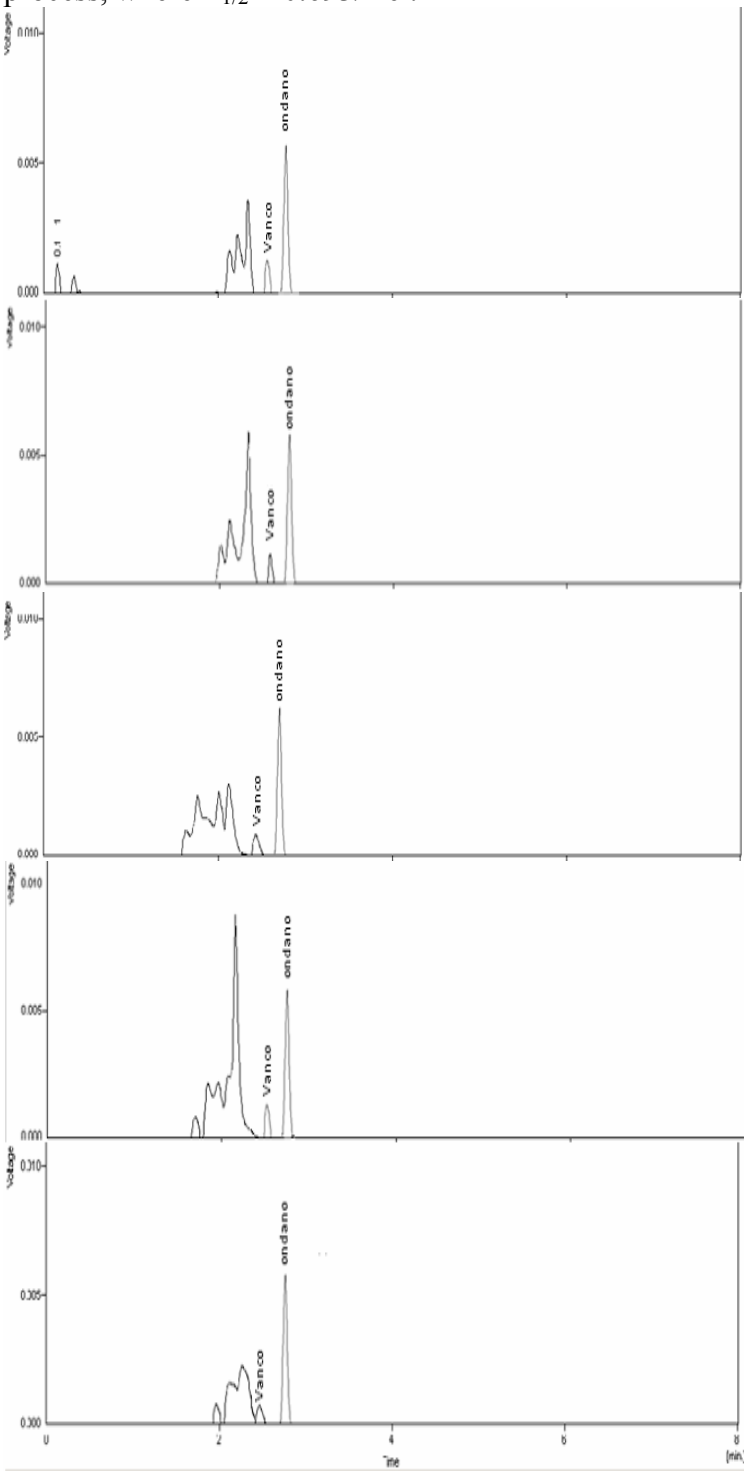

Figure 4. Five patient sample of spiked blood with 60µg/ml IS from SP1 to SP 5 for trough level AUC.

For patients who had not previously received vancomycin Vd was determined from the extrapolated initial concentration –Cmax (peak concentration) and Cmin (trough). For patients who had received vancomycin prior to the pharmacokinetic evaluation, calculation for Vd were modified to take into account the residual serum vancomycin conc. Existing from the previous dose: Vd= Dose/(Cmax-Cmin) where Cmax represented the calculated peak serum conc.following the first dose of vancomycin after the initiation of the SLED and Cmin represented the trough level from the vancomycin dose prior to the start of SLED therapy. Systemic clearance was determined from the calculated Kel and Vd : CLs = Kel X Vd .

Results

A total 11 patients (3 female & 9 male) participated at pharmacokinetic study. Patients demographics data are shown in Table 2 it was observe from mean age that max. patients of hospital is form upper age . Indian patients have the Avg. weight less then the Caucasian patients. From the chart of Table 3 of clinical data it was observed that the **bilirubin, SGOT and SGPT** are normal which indicate the patients in pharmacokinetic study has normal liver function or not so critical to give any variation much in study.

During the collection of the data we can able to manage 11 patient carring peaks as well as trough level of AUC is developed due to the limitation of correlation to contact/get patients collecting time schedules. It was well know that the creatinine clearance is co-related with the vancomycin clearance proportionally. In the formula CL= Crcl x 0.065 shows that systemic clearance of vancomycin –CL is 16.91± 6.99 with the respect to creatinine clearance. So, as the Crcl increase shows simultaneously increase in systemic clearance. And if the systemic clearance increase then the ($T_{1/2}$) of the drug , vancomycin , will reduce gradually . This measure the creatinine (Crcl) is inversely proportional to the half life ($T_{1/2}$).

Discussion

In the patients with normal renal function, the $T_{1/2}$ of vancomycin is ranges from 5.7 to 9.1 hrs . In patients with ARF receiving continuous renal replacement therapies, the vancomycin $T_{1/2}$ has ranged from 4.1 to 56.3 hrs .

We evaluate a mean vancomycin $T_{1/2}$ of 39.12± 6.81 hrs , which is longer than the $T_{1/2}$ observed in patients with normal renal function but falls within the range of half-life that have been reported for patient receiveing Continuous renal replacement therapies.

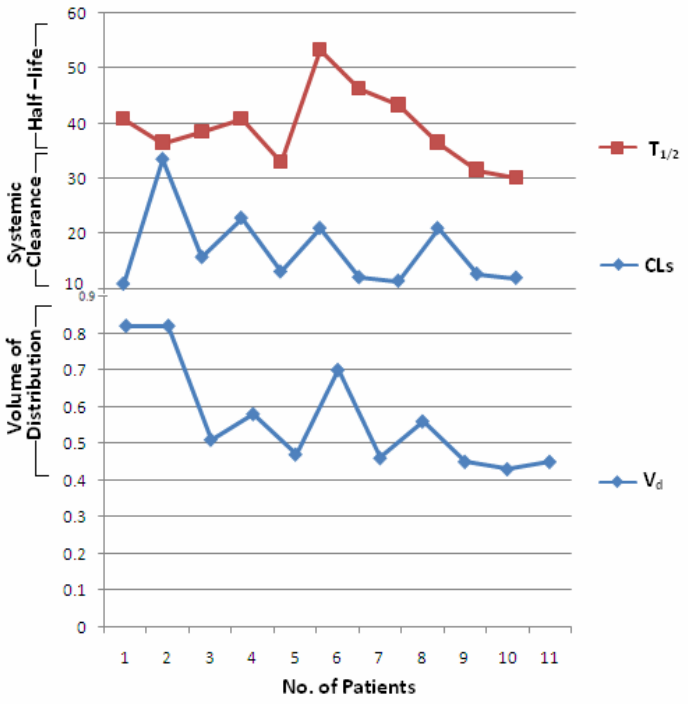

Figure 5. Correlation of Systemic Clearance(CLs); Half-life ($T_{1/2}$) and Volume of distribution (V_d).

We have observed a less degree of variability in the vancomycin half-life of our SLED patients. Vancomycin half life ranges from 30.13 to 53.3 hrs. Half –life is depends on the systemic clearance and possibly be explained by the variability that was observed in the vancomycin systemic clearance and distribution volume of the SLED patients in Figure 5.

In investigation the V_d of vancomycin ranged 0.43L/kg to 0.82L/kg and systemic clearance ranged 10.88 ml/min to 33.46 ml/min for the SLED patients . The mean of the systemic clearance is 16.91±6.99 and mean V_d is 0.57± 0.147. F_6 and F_5 membranes were mainly used in 10 patients with exceptionally one patient was employed with the F_7 membrane.

Here its observed that the creatinine clearance of the patients SP-1 is 14.45 ml/min and SP-2 is 44.55 ml/min but according to that the systemic clearance of vancomycin is 10.88ml/min and 33.46ml/min respectively shown relatively less proportional to each

other and thus the inversely proportion to the half –life i.e, 40.76 hrs and 36.47 hrs too, while the volume of distribution is same. These may be due to the volume of distribution or protein drug binding mechanism.

As finally after comparing the mean Half-life, volume of distribution & systemic clearance of the experimented values with the mean half-life of the reported Caucasian patients mention in Journal of Hospital Pharmacy, 2004[9]. We are getting reports of - Mean ± SD half-life, volume of distribution, and systemic clearance, were 43.1 ± 21.6 hours, 0.84 L/kg ± 0.17 L/kg, and 24.3 mL/min ± 8.39 mL/min, respectively in Journal of Hospital Pharmacy, 2004. Thus the t-test of the means were 0.5828, degree of freedom (df) is 20; standard error of difference is 6.829 and so, the two-tailed P value is 0.5665 i.e, P> 0.5 (this difference considered to be not significant).

Conclusions

In ethnic Indian SLED patients we found the $T_{1/2}$ mean ± SD of 39.12 ± 6.81 hrs compare to the Caucasian patients i.e, 43.1 ± 21.6 hrs. And the t-test and P-value is 0.5828 & 0.5665 respectively. Thus it was concluded that the half-life of ethnic Indians patients having less in compare to Caucasians but this difference is not so significant. The half-lives of ethnic 8 patient is less then 40 out of 11 patients.

Acknowledgments

The authors are thankful to CDL (Central Drug Laboratory), Kolkata, West Bengal, India for providing all internal standards and vancomycin and to Dr. T. K. Pal, Jadavpur University, Kolkata, West Bengal, India, for providing all facilities to complete this research work and for his helpful suggestions during the development and validation of the HPLC method.

References

1. Ryszard Międzybrodzki, Wojciech Fortuna, Beata Weber-Dąbrowska, Andrzej Górski. Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment. Postepy Hig Med Dosw. (online), 2007;61:461-465.
2. Cynthia Ginsberg, Stephanie Brown and Suzanne Walker. Bacterial Cell Wall Components. Glycoscience, 2008;6:1535-1600.
3. Lundstrom T, Sobel J. Antibiotics for gram-positive bacterial infections: vancomycin, quinupristin-dalfopristin, linezolid, and daptomycin. Infectious Disease Clinics of North America, 2008;18:651-668.
4. Vanholder R, Van Biesen W, Lameire N. What is the renal replacement method of first choice for intensive care patients? J Am Soc Nephrol. 2001;12:S40–3.
5. Marshall MR, Golper TA, Shaver MJ, et al. Sustained low-efficiency dialysis for critically ill patients requiring renal replacement therapy. Kidney Int.2001;60:777–85.
6. Amerling R, Caraiani NS, Hafeez T, et al. CVVHD with modified Fresenius 2008H: Initial experience . J of Am Soc Nephrol. 1988;9:166A.
7. Schlaeper C, Amerling R, Manns M, et al. High clearance continuous renal replacement therapy with a modified dialysis machine. Kidney Int. 1999;72:S20–3.
8. Das R, Pal TK , Nandy BC, Duttagupta S. Development of method of analysis for estimating the Vancomycin in blood plasma by RP-HPLC method: Application to *in vivo* Studies. Der Pharmacia Lettre, 2010;2:201-210.
9. John W. Ahern, Carl J. Possidente. Experience with Vancomycin in Patients Receiving Slow Low-Efficiency Dialysis. Hospital Pharmacy, 39:138–143.