

Pharmacokinetic Study of Frusemide in Healthy and Cirrhotic Indian Subjects

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ABSTRACT: Liver cirrhosis is associated with various complications such as ascites and fluid retention, progressing to development of hepatorenal syndrome, further compromising fluid elimination. Frusemide, a loop diuretic is normally administered to relieve fluid retentions. The kinetics of frusemide has not been conclusively reported in the three types of cirrhosis and among Indian subjects. The aim of the current study was to evaluate the kinetics of frusemide among healthy and Child's A, B and C cirrhosis and compare with earlier data.

24 cirrhotic were selected and classified according to the Child's-Pugh classification. 12 healthy male volunteers were screened and included in the study. 40 mg of frusemide was administered orally to both groups and blood samples were withdrawn at various intervals of time for a duration of 8 hrs. The amount of frusemide present in plasma was analyzed using HPLC. The volumes of distribution (V_d), area under curve (AUC), systemic clearance (C_L), maximum concentration (C_{max}), time for maximum concentration (t_{max}) in healthy volunteers were respectively 4.56 ± 0.15 L, 2258 ± 530.7 , 4.97 ± 1.67 L/h, 892 ± 49.4 ng/ml, 85.20 ± 7.49 mins. Corresponding values in Group A were 5.00 ± 0.31 L, 2471 ± 228.6 , 6.60 ± 2.90 L/h, 1021 ± 47.97 ng/ml and 88.25 V 2.12 mins; in Group B 7.73 ± 1.10 L, 4038 ± 154.7 , 8.84 ± 0.45 L/h, 1448 ± 43.20 ng/ml and 120 ± 1.89 mins; In group C cirrhosis 9.69 ± 1.32 L, 4085 ± 131.75 , 3.49 ± 1.40 L/h, 1551 ± 59.02 ng/ml and 185.7 ± 2.68 mins respectively. Significant differences at 1% and 5% were observed among the cirrhotic groups and between healthy v/s cirrhotic patients.

Data from current study do not correlate with earlier reports, carried mainly in Western population, due to possibly differences in instrumentation, etc but a possible genetic interplay should not be ruled out. Data from cirrhotic patients could not be effectively compared with earlier studies as kinetics of frusemide has not been conclusively been reported in the three categories of cirrhosis.

KEY WORDS: Pharmacokinetic, Frusemide, Liver cirrhosis.

INTRODUCTION:

Liver cirrhosis is one among the leading causes of death in the world and continues to represent a significant worldwide healthcare burden¹, estimated to have caused around 25,000 deaths in the United States in 1997². The etiologies of liver cirrhosis are various and among them are drug induced cirrhosis or cirrhosis due to certain

infections such as Hepatitis B and C. Pathological conditions such as biliary atresia, cystic fibrosis, gallstones, may also account for cirrhosis in certain conditions. Cirrhosis can be classified in three categories, according to the Child's- Pugh classification, depending on the severity of the disease¹.

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Liver cirrhosis is accompanied by a host of complications, specially the hepatorenal syndrome, causing retention of sodium and free water, decrease in renal perfusion and GFR³, thereby leading to edematous, and ascitic conditions.

Furosemide, a loop diuretic acting on the Na⁺/K⁺/2Cl⁻ symport in the luminal membrane of the ascending loop of Henle, inhibits the chloride and sodium reabsorption, but has no effect on the distal nephron⁴. It has been routinely used for the long term treatment of ascites in cirrhotic patients. Furosemide is an organic acid which is 96-98% plasma bound thereby limiting its delivery to the site of action. Pre-systemic metabolism of furosemide is carried out by the intestine while systemic clearance of furosemide is mainly performed by the kidneys with contributions from the intestine and the liver⁵. Since 30-50% of filtered sodium is reabsorbed in the loop of Henle using this transport system, furosemide has high natriuretic properties. At high dosage it may also increase sodium excretion up to 30%.

Presence of edema, due to renal malfunctioning, in cirrhosis, increases volume of distribution, altering drug bioavailability. In severe proteinuria, higher dosage of furosemide is required to achieve adequate free drug concentrations given that urinary albumin binds furosemide and reduces its effectiveness. Clinical non-responders tend to have a decreased fraction of loop diuretics excreted in urine, thereby predisposing them to the side effects of furosemide. Furosemide though not being significantly metabolized by liver may lead to severe side effects such as hyperuricaemia, electrolyte disturbances such as hypokalaemia and the hepatorenal syndrome observed in cirrhotic patients⁶. Furthermore, genetic traits, inter population variations and environmental factors may also significantly contribute to variability in drug response and disposition, thereby leading to a major clinical problem^{7,8}. Kinetics of furosemide have been earlier reported in both compensated and decompensated cases of liver cirrhosis, but reported studies were mainly carried out in Western population, using either intravenous furosemide or at a dose of 80-120 mg orally. However, no studies have been reported on the kinetics of 40 mg oral furosemide among Indian subjects.

The current study has been carried out among healthy and cirrhotic Indian subjects, with the aim to assess the pharmacokinetic parameters of

40 mg oral furosemide and to compare the generated kinetic data with earlier reported studies.

METHODOLOGY:

Patient recruitment: The study population included 24 cirrhotic patients. The cause and diagnosis of cirrhosis was identified based on clinical, biochemical and histological findings. Physical examination, nutritional status assessment including serum albumin, BMI, and Child's Pugh's Classification were determined. Recruited patients did not have any other diagnosed disease and no history of fresh internal bleeding. Prior to start of the study, patients were asked to stop furosemide intake 48 hrs prior to start of the test.

Volunteer Recruitment: 12 male volunteers in the age group of 24-29 years were recruited. Health status was ascertained by carrying out various biochemical tests, immunological tests and hematological examinations.

Collection of Study sample: Prior to start of the study, all subjects were fasted overnight but water intake was allowed. The cirrhotic and the healthy volunteers, all had 40 mg of oral furosemide and blood samples were collected in heparinised tubes at specified time intervals following drug administration, up to six hours. Blood samples were centrifuged at 4000 rpm for 5 mins, the supernatant withdrawn and stored in Eppendorf tubes at -20°C till the analysis.

Reagents: Furosemide (99.9%) purity was kindly donated by Aventis Pharma Ltd, India. Acetonitrile (HPLC grade) and Methanol (HPLC grade) were purchased from s.d fine chemical ltd India. Milli Q water was obtained from Millipore water systems, at a filter size of 0.22 µm. All solutions were degassed under vacuum before use. Glacial acetic acid (HPLC grade), HCl and NaOH pellets, were all brand products of Merck Ltd. The mobile phase consisted of a mixture of ACN-0.01 M sodium acetate, which was prepared by mixing ACN and degassed milli-q water in a ratio of 25:75. PH was adjusted to 5.0 with 4.0 M NaOH. The final mixture was degassed under vacuum before use.

Instrument Used: The HPLC instrument consisted of a 515 Waters (USA) pump and UV detector. The column was a 30 cm × 3.9 mm i.d. Bondapak C18 reversed phase column, particle size 10 µm (Waters Assoc). The whole system was controlled by a Millennium Version 2000 Unix computer programming system. The flow rate was adjusted at 2.0 ml/min at an ambient

temperature. The detection was followed by using a simple UV lamp detector calibrated at 280 nm. The pressure varied between 1812-2012 psi. Retention times were 5.0 mins and total run time was 10 mins.

Pharmacokinetic Parameters: Concentration time curves of frusemide in plasma were drawn, where values of Cmax and tmax were extrapolated. The area under curve (AUC) was determined by trapezoidal rule.

Value of β (elimination constant) was calculated from the elimination phase of the concentration time curve, using the following equation:

$$\beta = -2.3 \log (Y2-Y1)/(X1-X2)$$

(Where, Y2 and Y1 are the concentration values corresponding to the Y axis, and X1 and X2 are the time intervals corresponding to the X axis.)

Volume of distribution (V_d) was calculated using the following formula:

$$V_d = \text{Dose}/(\text{AUC} \cdot \beta)$$

Systemic clearance (Cl_s) was determined by:

$$Cl_s = \text{Dose}/\text{AUC}$$

RESULTS:

Area Under Curve (AUC): In healthy volunteers, the AUC, representing the concentration of frusemide present in plasma, was 2258 ± 530.7 ng/ml hr. In groups A, B and C cirrhosis, corresponding values were 2471 ± 228.65 , 4038 ± 154.7 and 4085 ± 131.75 ng/ml hr respectively. (Figure 1)

The difference in AUC between volunteers and group A patients was not statistically significant. However, AUC difference between healthy volunteers and groups B and C patients was statistically significant at 1% ($p < 0.01$). Significant differences were also observed in AUC for groups B and C vis a vis group A ($p < 0.01$). No statistical difference in AUC was observed between groups B and C of patients.

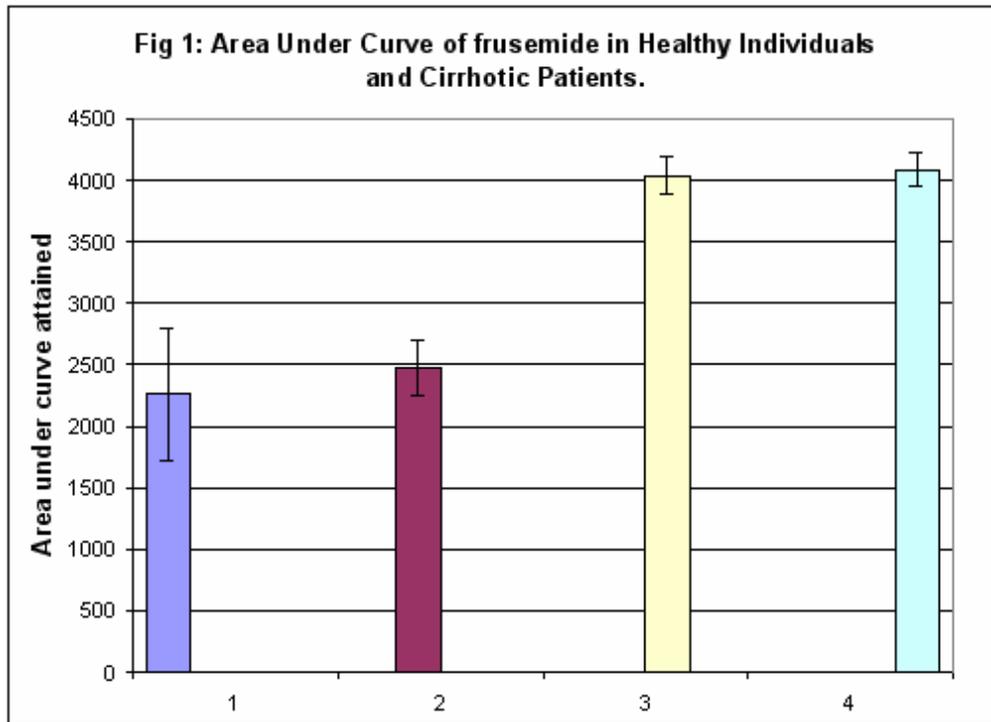


Figure 1: Figure representing the areas under the curves attained in Healthy individuals and cirrhotic patients. Area under the curve is representative of the concentration of the drug in plasma, calculated using the trapezoidal rule. 1: Healthy Volunteers; 2: Group A Patients; 3: Group B Patients; 4: Group C Patients.

Volume of distribution (V_d): Mean volume of distribution (V_d) in volunteers and group A, B and C cirrhosis were respectively 4.56 ± 0.15 L, 5.00 ± 0.31 L, 7.73 ± 1.10 L and 9.69 ± 1.32 L. There was no statistical difference between healthy volunteers and group A patients. However, statistical significance was observed between volunteers and B and C group of cirrhosis ($p < 0.01$). Significant differences were also observed between A and C group of cirrhotics at 5% ($p < 0.05$). No significant differences were observed between groups B and C. **(Figure 2)**

Systemic clearance (CL_s): In healthy subjects systemic clearance (CL_s) was calculated to be 4.97 ± 1.67 l/h, and 6.60 ± 2.90 , 8.84 ± 0.45 , and 3.49 ± 1.40 l/h respectively in Child's A, B and C cirrhosis. Clearance increased significantly among cirrhotics in comparison to volunteers ($p < 0.05$). However in C group of cirrhosis, systemic clearance decreased considerably. **(Figure 3)**.

Mean Maximum Plasma Concentrations of Frusemide (C_{max}): Maximum plasma frusemide concentration attainable in healthy volunteers was calculated at 892 ± 49.4 ng/ml, whereas in the various categories of Childs classification, they were respectively 1021 ± 47.97 , 1448 ± 43.20 , 1551 ± 59.02 ng/ml. Whereas no significant difference was observed between healthy subjects and A group of cirrhosis, B and C groups of cirrhosis demonstrated significant difference in C_{max} values vis a vis volunteers ($p < 0.01$). **(Figure 4)**

Time taken to attain Maximum Plasma Concentration (t_{max}): t_{max} values in healthy volunteers and in groups A, B and C of cirrhosis were as follows: 85.20 ± 7.49 , 88.25 ± 2.12 , 120 ± 1.89 and 185.7 ± 2.68 minutes respectively. Group A and volunteers displayed no significant difference in t_{max} values, however, significant differences at 1% were observed between B and C group of cirrhosis. **(Figure 5)**

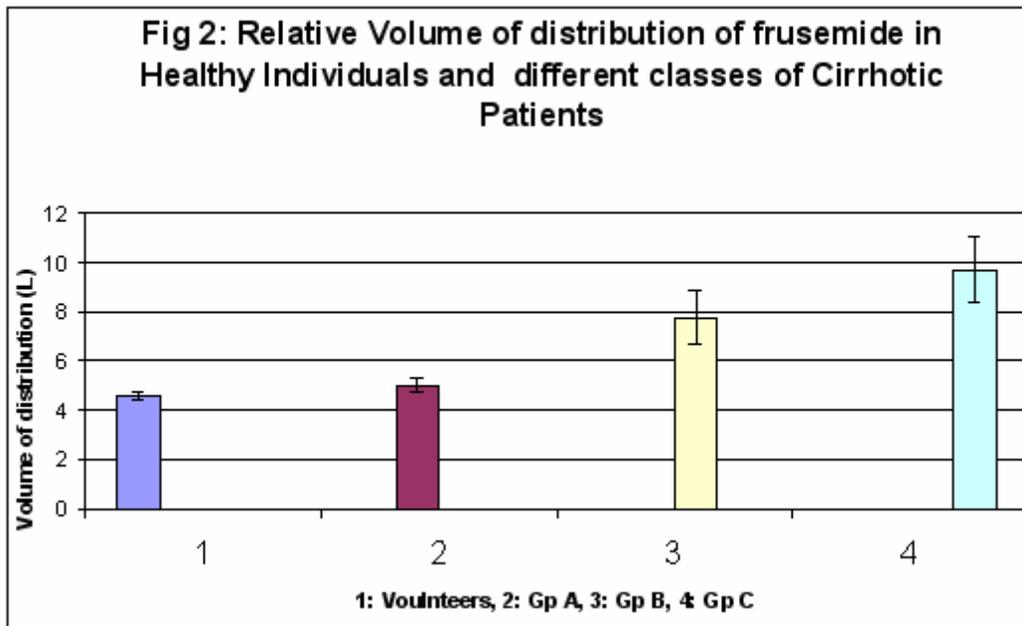


Figure 2: Relative volume of distribution of frusemide in volunteers and different categories of cirrhosis. Volume of distribution calculated gives an indication of rate of cirrhosis. 1: Healthy Volunteers; 2: Group A cirrhosis; 3 Group B cirrhosis; 4: Group C cirrhosis.

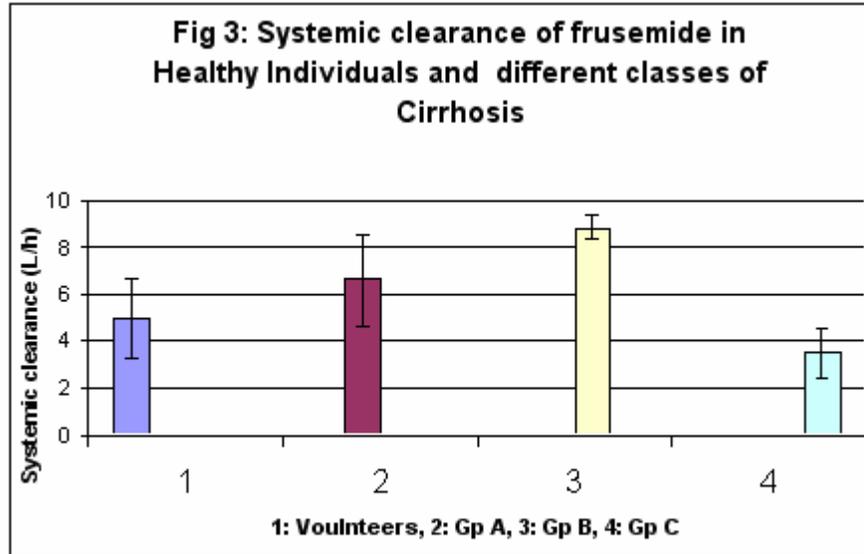


Figure 3: Systemic clearance was calculated using the formula, Clearance indicative of the liver functions. Significant differences was observed between healthy individuals and Group B and C cirrhotic patients

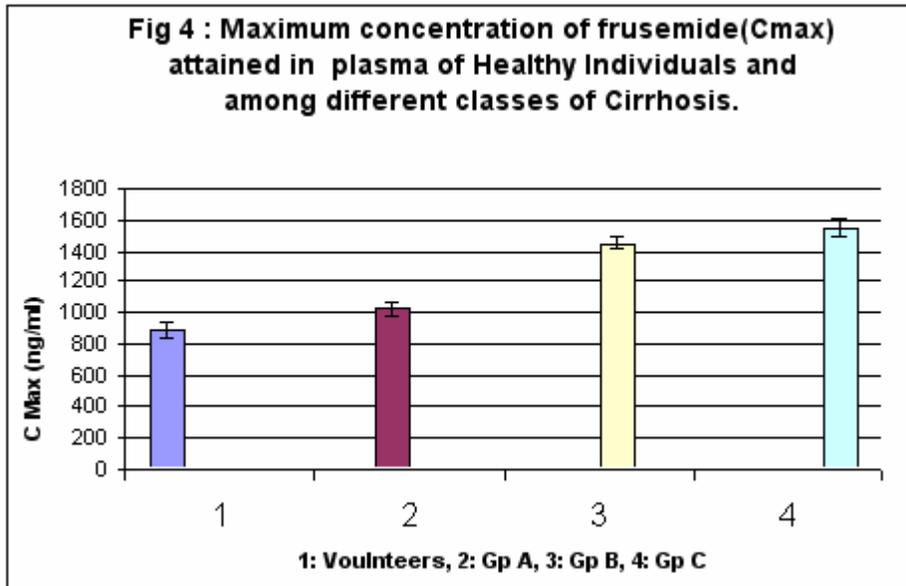


Figure 4: Maximum plasma concentration of frusemide was calculated using the formula mentioned. Significant difference at 5% was observed between healthy individuals and cirrhotic patients. An inter-group difference among cirrhotic patients was also observed.

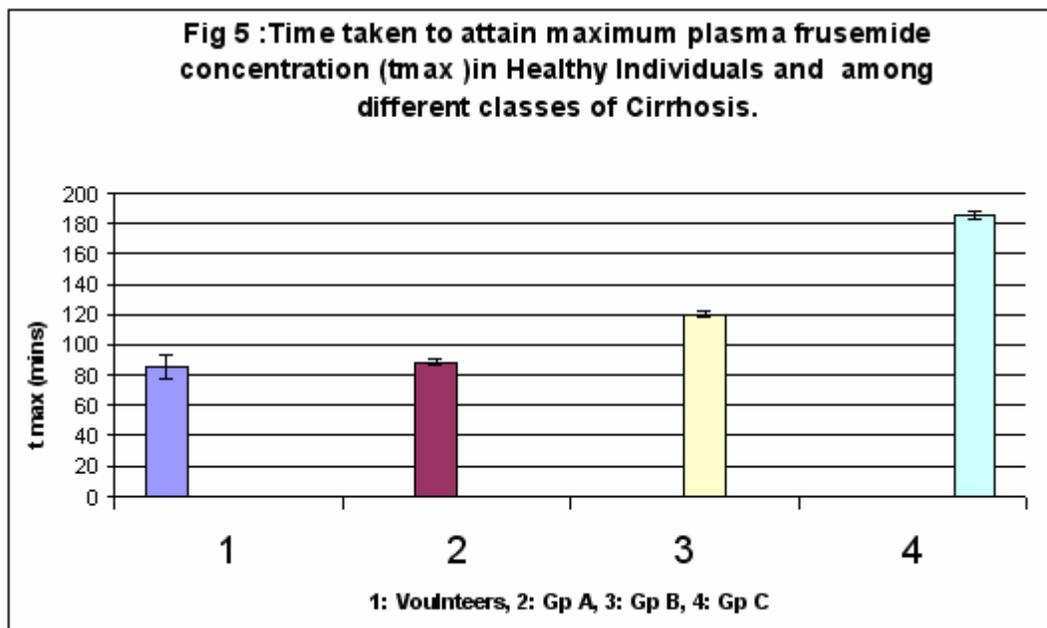


Figure 5: t_{max} values have been calculated using the formula. t_{max} values were significantly different between the healthy individuals and the different classes of cirrhotic patients.

DISCUSSION:

Liver cirrhosis highly influences the dynamic state of the human body. Earlier reports have suggested altered kinetics of a spectrum of drugs such as fluconazole⁹, omeprazole¹⁰ and a host of other drugs, which are generally administered during cirrhosis. Several kinetic studies in cirrhosis have been conducted, but there has been no conclusive study on the three types of cirrhosis and also kinetic studies among Indian population. The objectives of this study was to shed light on the kinetics of frusemide among healthy and three types of cirrhosis among Indian population and evaluate any possible deviations from the reported studies.

The kinetics of frusemide among Indian volunteers was not in accordance to previously reported data generated from healthy individuals. Earlier reported t_{max} values ranged between 60-70 mins¹¹ and 100-150 mins after 40 mg and 80 mg of oral frusemide respectively in healthy volunteers. In the current study, the t_{max} value among Indian volunteers was observed to be 90 mins. The volunteers had fasted overnight, but some had breakfast after drug administration. Since food tends to retard kinetics of frusemide¹², this might have possibly led to observed increase in t_{max} value. However, the increase in t_{max} could also be due to slow absorption kinetics amongst Indian Subjects.

Current calculated C_{max} value (0.904 ± 0.452 ng/ml) in healthy volunteers was lower than earlier reported values¹¹, which could be explained to the difference in administered dose of frusemide ($40 \text{ mg} \text{ v/s } 80 \text{ mg}$). Bioequivalence could also be affected by the nature of formulations used¹³. Correspondingly, mean area under curve ($2.23 \pm 0.5 \mu\text{g/ml h}$) were lower among Indian volunteers in view of the lower C_{max} and t_{max} values. The mean half-life values reported are divided on this issue. Higher mean half-life values have been reported elsewhere, but studies conducted by **Rupp et al**¹⁴ and **Cutler et al**¹⁵ have reported similar values as observed in our study. Age¹⁶, study design¹⁷, analytical methods and drug formulation¹⁸ may also alter the course of the drugs in the body, thereby explaining the difference in the observed difference in trend.

Among the cirrhotic groups, observed kinetic parameters were not in accordance to earlier reported values. The C_{max} and t_{max} in our study increased proportionally in categories A, B and C of cirrhosis vis a vis healthy volunteers. The increase in t_{max} is due to presence of edema of the gut along with changed motility, which is predominant in cirrhosis¹⁹. It is also reported that cirrhosis causes prolongation of gastric emptying time (GET), leading to delayed absorption and thereby raising C_{max} and t_{max} ^{20,21}. However,

current C_{max} and t_{max} could not be compared to earlier reports, due to non availability of data on these parameters.

Current observed mean AUC increased in parallel with severity of disease. The increase in AUC among the three type of cirrhosis could be explained due to decreased serum albumin level, hence affecting its binding to frusemide. The increase in AUC level could also be due to decreased systemic clearance, responsible for the observed increase in volume of distribution and half-life. The drug clearance in Group C is also further significantly reduced owing to a decrease in systemic clearance. The volume of distribution reported earlier^{22,23,24} correlated to some extent to that observed in our group C patients. This indicates that earlier studies might have been specifically conducted among Child's C cirrhotics. The mean albumin levels among cirrhotics (3.55 ± 0.34 group A, 3.08 ± 0.22 group A, and 2.54 ± 0.22 group C mg% respectively) in the current study were lower in comparison to volunteers (4.75 ± 0.25). It has been earlier reported that a decrease in protein binding²⁵ causes an increase in V_d , thereby justifying the increase in the volume of distribution among cirrhotics. Similar trend in

volume of distribution has been reported with lidocaine²⁶, diazepam²⁷ and ampicillin²⁸ among cirrhotic patients. Current V_d increase in our group C patients, could also be due to the possible development of ascites during the course of the study. Our data of V_d correlate with earlier reports, suggesting that earlier studies might have explored kinetics of frusemide in group C patients. Most data sources available, do not distinguish between the types of diabetes, thereby rendering comparison with earlier studies difficult.

Reports on the systemic clearance (C_L) illustrative of the drug clearance from the body were variable (**Table 1**). Whereas some studies have reported decreased frusemide clearance among cirrhotic patients^{22,23,29}, others have reported high clearance rate in compensated cirrhosis, and no difference in decompensated groups²⁴. Earlier reports did not mention about the stages of cirrhosis, thereby making it difficult to compare currently calculated values. As volume of distribution is inversely proportional to C_L , our observations are justified by the observed increase in volume of distribution with a paralleled decrease in systemic clearance among the three types of cirrhosis.

Table 1: Summary of reported kinetics of frusemide in cirrhotic patients

Pharmacokinetic parameters	Dose (mg) /Administered Route	t_{max} (min)/ C_{max} (μ g/ml)	$t_{1/2}$ (mins)	V_d (L)	C_L (L/h)
Gonzalez et al ²²	40/iv	NA	81.0 \pm 8.0	10.7 \pm 2.2	6.6 \pm 1.74
Verbeeck et al ²³	80/iv	NA	79.4 \pm 18.3	12.1 \pm 1.3	8.52 \pm 0.96
Traeger et al ²⁴	40/iv	NA	65.4 \pm 14.4C* 110 \pm 32.4D*	6.92 \pm 0.75C* 11.42 \pm 3.84D*	4.83 \pm 1.32C* 2.64 \pm 1.02D*
Sawhney et al ²⁹	80/iv	60-240	129 \pm 75	12.0 \pm 3.5	7.2 \pm 2.16
Current Study					
Child's A	40/oral	90/1.02 \pm 0.47	45.45 \pm 0.133	5.00 \pm 0.32	6.60 \pm 2.90
Child's B	40/oral	120/1.44 \pm 0.43	53.76 \pm 0.047	7.73 \pm 1.1	8.84 \pm 0.45
Child's C	40/oral	180/1.55 \pm 0.59	64.4 \pm 0.17	9.69 \pm 1.32	3.48 \pm 1.40

Currently observed increase in systemic clearance among A and B groups of cirrhosis vis a vis healthy volunteers indicates compensated stage. Compensated stage is expressed by a compensatory increase in renal excretion of unchanged drug, due to insufficient detoxification capabilities of the liver²⁴. The relatively decreased systemic clearance of the drug in group C cirrhosis may also be the result of higher age of patients in that category and hence their associated dwindling renal functions

(Nielsen 1973). It is also reported²⁵ that cirrhotics have essentially no non renal frusemide elimination.

CONCLUSION:

Current kinetic parameters among healthy Indian volunteers do not correspond to the earlier reports carried out mainly in Western countries. Such differences may be explained on various basis such as the difference in techniques, aging, sample selection, etc as explained above, but a

certain genetic interplay influencing drug disposition should not be ruled out. Genetic differences is becoming commonly known and there are various approaches to create a 'tailor made' to reduce economic drug and curtail side effects.

Among Indian cirrhotic patients, the data obtained could not be effectively compared to earlier studies due to lack of conclusive data on the three types of cirrhosis. Thus our study was a pioneer study among the Indian subjects and also in the various categories of cirrhosis. Moreover, kinetic trend among A, B and C type cirrhosis could not be compared to earlier reported trends, which could possibly be influenced by genetic factors.

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