FIRST DOSE RESPONSE OF TREATMENT OF THREE FORMULATIONS OF INTERFERON ALFA IN PATIENTS USING LIMITED SAMPLING STRATEGY

RUKHSANA SHER,[1] NAFEESA FATIMA,[2] ARSHAD K. BUTT[3] AND ANWAR A. KHAN[3]
Departments of [1]Pharmacology, [2]Hematology and [3]Gastroenterology
Shaikh Khalifa Bin Zayed Al-Nahyan Medical College, Shaikh Zayed Medical Complex and PGMI, Lahore – Pakistan

ABSTRACT

Aim: The study was conducted to observe the comparative response of three commercially formulations of interferon alpha 2_b in three groups of patients with chronic hepatitis C. The objective was to see best response after first dose of interferon Alfa 2b.

Methods: This was a quasi – experimental study in which patients of both sexes between ages 20 – 49 were randomly allocated to receive three different formulations of interferon alpha 2b in three groups, I, II, and III, after giving them first dose of three million units of interferon alpha 2b subcutaneously. Sixty patients were selected in three groups according to the formulation injected. Blood samples were collected at 00, 08, 20 hours according to limiting sampling strategy. All samples were analyzed by ELISA.

Results: The response of serum drug level was different between three groups. Statistically significance differences were observed between group I and II, and group I and III, based on ANOVAs and t-test. Comparison between three groups was significant. Almost all subjects (98%) reported typical mild interferon side effects (flu – like symptoms, headache).

Conclusions: Different formulations have variable response of serum drug level

INTRODUCTION

Viral hepatitis may start and resolve quickly (acute hepatitis), or cause long – term disease (chronic hepatitis). In some instances, progressive liver failure, or even liver cancer may result.[1] The global prevalence of hepatitis C virus infection is approximately 3% (170 million people).[2] About 5 million people are infected with hepatitis B virus and about 10 million people harbor the hepatitis C virus in Pakistan.[3] Management of chronic hepatitis in Pakistan carries substantial social impact like fear of tolerability of the drug due to its side effects and the most commonly its affordability.[7]

Treatment strategies which most of the general population employed are the visit to faith heelers, herbal treatment and pharmaceutical therapy.[8] Pharmaceutically interferon in combination with ribavirin is used for the treatment of chronic hepatitis C patients.

There are almost sixty preparations of alpha interferon are available in Pakistan, imported from different countries like China, Korea, Switzerland, Cuba, and Argentina etc. and is an expensive preparation so it is essential to see the response of drug in order to choose the best product on the basis of serum level of the drug.

This study will decide the response of locally available interferons and help in selecting the best drug with the most favorable serum response.

PATIENTS AND METHODS

It was a Quasi – experimental study. Patients were enrolled with chronic hepatitis C from hepatitis clinic Shaikh Zayed Hospital, Lahore. Study was completed in six months from March 2008 – September 2008.

Sample Size

Sixty patients of chronic hepatitis C virus positive by ELISA, this was confirmed with PCR and genotype was included in this study. The sample size was calculated using a computer SPSS version 15 was used to analyse the data.

Thirty males and 30 females who were hepatitis C positive were confirmed on PCR. They were randomly allocated to the three subgroups.

1. Uniferon (Getz).
2. Anferon (CCL).
3. Ceron. (Biocare).

The sampling technique was purposive non probability sampling. The inclusion criteria included confirmed cases of chronic hepatitis C ELISA and PCR, age between 20 – 49 and either gender.

Patient Exclusion Criteria

- With co morbidity of diabetes mellitus, hypertension, hepatic, renal, cardiac failure, and cancer. Those on special drugs like steroids, immunosuppressant and herbal medications, and addicts of alcohol or other drug abuse.

Data Collection Procedure

A group of sixty cases of chronic hepatitis C virus positive by PCR test were identified and requested to join the study group voluntarily. They were divided into three subgroups, uniferon, anferon, and ceron, of twenty in each on random allocation basis.

An informed consent was obtained from all of them for allocating them to different subgroups and using their data in my research. The study was approved by the Institution Review Board, Sheikh Zayed Medical Complex Lahore. Demographic information like name, age, sex, address, height, and weight were recorded.

History of illness was explored regarding types of symptoms, duration and severity.

Investigations, LFTs, CBC, and genotyping and HCV RNA PCR (Quantitative) were performed.

The patients were given first injection of three million units of alpha interferon 2b (three different products as Uniferon, Anferon, and Ceron) administered subcutaneously to the subgroups.

Venous blood (5 ml) was drawn for non heparinised vacutainer for plasma interferon level, at 8 and 20 hours after the first injection in accordance with limited sample strategy.

Blood samples for plasma interferon level were immediately centrifuged at 4000 rpm / minute for 5 minutes. Plasma was removed and frozen instantly at -80°C and maintained in the frozen state till analysed.

Plasma alpha interferon concentrations were determined by DYNEX Human ELISA reader, Best 2000 ELISA system. The procedure followed the instructions of the manufacture, BIOKIT, S.A. 08186 Lica d'Amunt, BARCELONA – SPAIN.

Data Analysis Procedure

The serum level of interferon was performed at the time of first injection.

The collected information was entered in SPSS, version 15 and analysed through it. Demographic covariates were presented as mean and standard deviation (SD). The sex distribution was presented as proportional. The

Table 1: *Males showing Plasma level at 8 and 20 hours after Inteferon Alpha 2 B (IU/ML).*

No.	*Group 1*		*Group 2*		*Group 3*	
	After 8 Hours	*After 20 Hours*	*After 8 Hours*	*After 20 Hours*	*After 8 Hours*	*After 20 Hours*
1.	87.43	5.58	41.14	6.68	41.70	3.36
2.	78.24	3.63	94.74	00	56.21	4.75
3.	43.23	4.23	76.99	3.26	66.81	3.35
4.	78.95	3.69	71.12	00	86.12	3.90
5.	75.58	3.56	55.87	6.29	79.30	3.33
6.	61.55	3.71	26.36	4.96	49.99	2.16
7.	90.15	3.67	42/26	00	58.69	3.76
8.	53.25	3.4	26.90	3.9	33.94	00
9.	75.18	3.26	56.20	3.41	32.20	00
10.	75.21	4.5	70.60	3.12	68.55	9
Mean	71.4	5.75	60.4	4.61	57.35	4.52
S.D.	11.1	0.8	10.1	0.8	9.9	0.8

Group 1 Uniferon Group 2 Anferon Group 3 Ceron α

Table 2: Fem*ales showing Plasma level at 8 and 20 hours after Inteferon Alpha 2 B (IU/ML).*

No.	*Group 1*		*Group 2*		*Group 3*	
	After 8 Hours	*After 20 Hours*	*After 8 Hours*	*After 20 Hours*	*After 8 Hours*	*After 20 Hours*
1.	60.65	4.2	45.56	3.2	45.65	3.4
2.	49.58	4	35.68	4.6	30.95	4.3
3.	70.38	6.2	50.53	3.8	55.65	3.6
4.	50.48	8.1	45.13	9.6	40.28	6.7
5.	48.5	12.3	35.28	5.1	35.68	5.1
6.	40.38	3.4	36.18	5.7	40.28	8.5
7.	55.68	5.2	45.96	7.8	43.18	9.5
8.	48.5	10.1	46.38	5.1	38.56	4.6
9.	50.4	8.9	45.28	3.2	40.48	3.5
10.	50.38	7.5	45.68	408	42.68	3.3
Mean	52.5	7.0	43.1	5.3	41.3	4.9
S.D.	8.7	1.3	7.3	1.1	7.2	1.1

Group 1 Uniferon Group 2 Anferon Group 3 Ceron α

past history of any significant medical illness was presented on frequency tables. Presence or absence of any co morbidity was present or absent. Initially one way ANOVA were applied to identify any significant difference between three groups. Any difference observed between the groups for their variables were tested for significance by applying t-test for quantitative variables and chi square test for qualitative data. Descriptive analysis of socio-demographic symptoms, signs and of investigations were carried out.

Table 3: *Mean weight, weight and body surface area of both males and females of three subgroups.*

Parameter	*Group 1*	*Group 2*	*Group 3*
Weight in kg	53.74 ± 8.9	54.22 ± 9.0	53.44 ± 8.90
Height in cm	160.52 ± 26.75	161.02 ± 26.83	159.02 ± 26.50
Body urface area (M^2)	1.547 ± 0.257	1.558 ± 0.259	1.543 ± 0.257

Group 1 Uniferon Group 2 Anferon Group 3 Ceron α

Males Plasma Level

	UNI 8	*UNI 20*	*ANF 8*	*ANF 20*	*CER 8*	*CER 20*
Mean	71.37	5.75	60.44	4.61	57.35	4.52

Females Plasma Level

	UNI 8	*UNI 20*	*ANF 8*	*ANF 20*	*CER 8*	*CER 20*
Mean	50.59	6.99	45.16	5.29	41.33	4.91

Statistical Analysis

Using the SPSS version 15, all numerical variables were represented as mean ± SEM on the basis of the test described below: Since the study involved three groups, the quantitative values were subjected to one – way ANOVA.

After getting some significance the difference between groups I – II, I – III and II – III were independently compared with t-test of significance.

The independent T-test was used to analyse between two groups i.e. Uniferon: Anferon. Ceron: Anferon. Ceron: Uniferon.

The parameters of the three sub-groups were subjected to statistical analysis using one – way ANOVA. P-value was highly significant between I and II and I and II. Post – hoc method performed to compare parameters between three subgroups, factorial analysis of gender and sample time when interact they give us significant results. Gender difference between males and females of three subgroups was significant with p-value < 0.001. The 95% confidence interval assigned to the mean parameters. p-value < 0.5 was considered significant for all analysis.

Plasma level of males and females were explained in Table 1 and 2 which revealed that the maximum plasma level was seen in group II, was 94.74 among males, in females the highest plasma level was 70.38 of group I at 8 hours, the difference in plasma level in females was explained in Table 2. The minimum level observed in group II and III at 20 hours was undetected among two patients each, the lower limit of Elisa essay was 3 IU. Samples obtained before the interferon alpha 2b were below the limit of quantification for all patients suggesting that endogenous interferons do not interfere significantly with the pharmacokinetic study. From samples obtained at 0, 8 and 20 hours post dose the alpha interferon administration, it was possible to obtain precise and unbiased individual plasma interferon assay in 60 patients.

Patients	*C max (IU/ml)*
Uniferon (20)	60.98 ± 0.1
Anferon (20)	52.8 ± 8.8
Ceron (20)	49.34 ± 8.2
P-value	*<0.01

*Between I and III, I and II

The maximum plasma concentration of males in three subgroups at 8 hours was 71.37, 60.44, and 57.35 of group I, II and III respectively.

The maximum plasma concentration of females in three subgroups at 8 hours was 50.59, 45.16, and 41.33 of group I, II and III respectively.

The maximum plasma concentration of males at 20 hours was 5.15, 3.76 and 9.88 between groups I, II and III respectively at 20 hours was negligible. The plasma concentration of females at 20 hours was 6.99, 5.29 and 4.91 between groups I, II and III respectively at 20 hours was negligible.

Plasma level and comparison of plasma level between the three subgroups which explained that group I males and females had maximum plasma interferon level 60.98 ± 10.1 as compared to group II 52.80 ± 8.8 and III 49.34 ± 8.2 respectively.

The difference of mean plasma level between three subgroups was significant among group I and group II, the difference between group II and three was insignificant.

Statistical analysis showed
One way ANOVA and Significance between groups
Group I and II, $p < 0.01$
Group I and II $p < 0.01$
Group II and III $p > 0.05$.

ACKNOWLEDGEMENTS
The authors are grateful to the administration of Sheikh Zayed Hospital Complex for allowing them to carry this work.

REFERENCES

1. Ryder SD, Irving WL, Jones DA, Neal KR, Underwood JC. Progression of hepatic fibrosis in patients with hepatitis C: a prospective repeat liver biopsy study. Gut 2004; 53: 451-5.
2. The epidemiology of hepatitis C infection in the United States. J Gasteoenterology, 2007; 42: 513-21.
3. Raza SA, Clifford GM. Worldwide variation in the relative importance of hepatitis B and hepatitis C viruses in hepatocellular carcinoma: Br J Cancer. 2007; 96: 1127-34.
4. The Health Foundation Pakistan (Viral Hepatitis Epidemiology 2006) hepatology 36: 227-42.
5. The Health Foundation Pakistan (Viral Hepatitis Epidemiology 2006) hepatology 36: 227-42.
6. Idress M and Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission 2008.
7. Wiela – Hojenska A, Orzechowska – Juzwenko K. Bioavailability and its significance in pharmacotherapy. Pol Merkur Lekarski, 2003; 14: 89-93.
8. Brunetto MR, Colombatto P, Bonino F. Personalized therapy in chronic viral hepatitis. Mol Aspects Med, 2008; 29: 103-11.