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Varied Virological Response of Patients with Chronic Hepatitis C against the Treatment of Pegylated Interferon- α and Ribavirin

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ABSTRACT

INTRODUCTION : *Hepatitis C Virus (HCV) is the leading cause of death throughout the world. The standard of care for the treatment of chronic hepatitis C is combination therapy with Pegylated Interferon (PEG-IFNα 2a) and Ribavirin (RBV). There currently exists no systematic explanation for these genotype-specific differences in clinical outcome. Furthermore, whether factors that govern outcome for one genotype play a similar role in other genotype remains to be fully explored. Hence, the present study was taken in consideration of the factors emphasizing their impact on the sustained virological response (SVR) against HCV genotypes.*

METHODOLOGY : *A total of 50 patients (Age, Mean: ±SD 42.53 ± 12.6) having chronic hepatitis C genotype 3 and genotype 1 who showed positive result for HCV-RNA for more than 6 months were treated with combination therapy of PEG-IFNα 2a and RBV. All the patients were followed up for 48 weeks of post treatment and varied virological response was recorded in respect to the HCV genotypes, subtypes and biological parameters.*

RESULTS : *In present study, we have observed that males had a better SVR and EVR as compared to females in both the genotypes (genotype 1 and genotype 3) and among the non responders there were less males as compared to females. It was also seen that there were less females who showed EVR and SVR as compared to males.*

CONCLUSION : *Our study has demonstrated that EVR, RVR, NR and most importantly SVR are important factors for the achievement of complete virological response against HCV genotypes and subtypes.*

KEYWORDS : *Virological response, HCV, Pegylated Interferon-α and Ribavirin*

I. INTRODUCTION

Nearly 170 million people worldwide are chronically infected with HCV [1]. In the United State, HCV is the leading cause of hepatocellular carcinoma and the leading indication for liver transplantation [2].The standard of care for the treatment of chronic hepatitis C is combination therapy with PEG-IFNα 2a and RBV. PEG-IFN is a synthetic variant of interferon-α (a naturally occurring cytokine) whose endogenous role is to activate the innate immune response within the host. Injected PEG-IFN is hypothesized to function by mimicking the natural cytokines. RBV is a nucleoside analog and is thought to act through a combination of other modalities [3, 4]. PEG-IFNα2a offers significantly enhanced SVR in all patients, regardless to HCV genotype and viral load. The ability to predict the absence of SVR against HCV at molecular level will be a useful clinical tool. The combination therapy with Peg-IFNα 2a and RBV provides a considerable clinical advantage over conventional therapy [21]. Large clinical trials of PEG-IFN/RBV therapy have revealed significantly different response rates for the various HCV genotypes.

There are six major HCV genotypes, named genotype 1 to 6 in which genotype 2 has been showed the most responsive with a SVR rate of >80%. Studies have also suggested that it is reasonable to treat some patients infected with this genotype for only 12–16 weeks. Conversely, the most prevalent genotype worldwide, genotype 1 is the least responsive [5, 6, 7]. The SVR for patients infected with genotype 1 is less than 50%. Current guidelines recommend 48 weeks of therapy for this particular genotype; shorter courses of therapy have been demonstrated to be sub-optimal [8]. There currently exists no systematic explanation for these genotype-specific differences in clinical outcome [4, 9, 10]. It is assumed that genotype-specific clinical response rate is the result of a confluence of host and viral factors and remains a challenging area for further promising investigations. Furthermore, whether factors that govern outcome for one genotype play a similar role in other genotype remains to be fully explored. Based on the genotype variability, viral load, IFNα dose, and the treatment duration, SVR rate upto 55% has been achieved in patients with HCV other than the HCV genotypes 1 [11]. Whereas, SVR rate of 38% to 67% had been achieved in patients with genotype 3 based on the dose of IFNα [12, 13]. Various comparative study results as per the existing biomedical literature available on IFNα monotherapy versus PEG-IFNα shows a SVR rate of 24% to 46% and 38% to 68% respectively [14, 15]. Furthermore, biomedical researchers have compared the efficacy of the combination therapy of PEG-IFNα dose of 3 million units three times per week. SVR rate of patients with HCV genotype 3 were 21% to 32% [16, 17] for IFNα and 38% to 45% for PEG-IFNα [15, 18]. In subtypes of HCV genotype 3 patients with low baseline viral load have showed almost 58% of SVR those who were treated with PEG-IFNα [18]. However, currently the standard treatment for patients with HCV genotype 3 is a combination therapy of PEG-IFNα and RBV for 24 weeks attaining SVR rates of upto 80% [19] and for 48 weeks in genotype 1 patients attaining SVR of 40% to 50% [19]. Patients with HCV genotype 3 and 1 were treated with a schedule (RBV-600mg/day or 1000-1200mg/day i.e,. 1.5mg/kg bogy weight) and PEG-IFN α2a at a dose of 180mcg (15mg/kg body weight) as per the guidelines of National Institute for Clinical Health and Excellence.

Since the combination therapy with RBV increases the rate of side effects, the discontinuation rate is more frequent and patient's haemoglobin concentration decreases. Independent prognostic factors for SVR include the viral load, viral genotype, absence of cirrhosis or fibrosis, age and gender. There remains a probability that the SVR rates could be influenced by considering these prognostic factors. Hence, the present study was taken in consideration of all the above factors emphasizing their impact on the SVR against HCV genotype 1 and genotype 3.

II. MATERIALS AND METHODS

2.1 Patient's selection

A total of 50 patients (age, Mean±SD: 42.53 ± 12.6) attending the centre (from January 1994 to December 2010) having chronic hepatitis C genotype 3 and genotype 1 who showed positive result for HCV-RNA for more than 6 months were treated. All of them were treated with combination therapy of PEG-IFNα 2a and RBV. Chronicity was receorded by the longitudinal observation and presence of advanced clinical liver disease in patients who refused to undergo liver biopsy (**Fig. 1**).

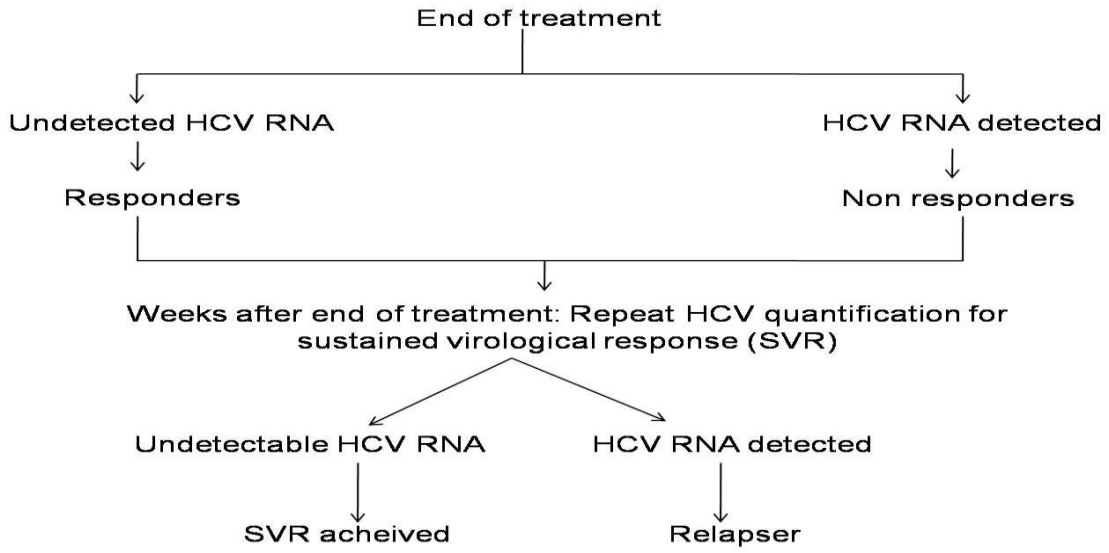

Fig. 1: Study design for SVR against HCV genotypes and subtypes using demographical, clinical and molecular parameters

Following inclusion criteria's were taken in account for screening of the subjects:
a) patients with chronic Hepatitis C by clinical criteria and/or histopathology,
b) patients with positive HCV-antibody and positive HCV-RNA, and
c) Patients with HCV genotype 3 and genotype 1 infection.

The patients with decompensated liver diseases, improperly controlled diabetes, active auto immune disorders, alcohol or intravenous drug abusers, high levels of serum alpha-feto protein concentration, past history of psychiatric illness, anemia, thrombocytopenis, and pregnancy excluded from the study. Further, the patients with Hepatitis A Virus (HAV) in active form, Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infection were also excluded. The study was based and conformed upon the ethical guidelines of 1975 declaration of Helenski and was approved by the Institutional Ethics Committee of Deccan College of Medical Sciences, Hyderabad. Before collection of the samples all the patients were informed well and written informed consent was taken.

2.2 Detection of serum HCV-specific RNA by RT-PCR

The extraction of viral RNA from serum samples was done using QIAamp Viral RNA Kit (Qiagen, Germany) according to the manufacturer's instructions. Complementary deoxyribonucleic acid (cDNA) was prepared using viral RNA specific reverse primer and reverse transcriptase II (Fermentas, Burlington, Canada). 5ng of cDNA was used for reverse transcription quantitative polymerase chain reaction (RT–qPCR) analysis using Taqman chemistry in StepOne Real-Time PCR (Applied Biosystems, CA, USA).

2.3 Determination of HCV genotypes and subtypes

The samples tested positive for HCV RNA, genotyping and subtyping was done by Innolipa HCV II line probe assay (Innogenetics, Ghent, Belgium).

2.4 Treatment regimen

180mcg (15mg/kg body weight) of PEG-IFNα 2a and 600 mcg (1.5mg/kg body weight) of RBV were given to the patient once a week for about 24-48 weeks as per the dose approved by Roferon-A, F. Hoffmann-La Roche, Basel, Switzerland. Then after the period of 24-48 weeks therapy, the end of treatment (EOT) was used to define the SVR against HCV infection.

2.5 Monitoring of the patients

The patients were observed at 2^{nd}, 4^{th}, 6^{th} and 8^{th} weeks of their treatment at an outpatient setting and then every 4^{th} week thereafter during the treatment. After the EOT, the follow-up assessments were made at every 24^{th} and 48^{th} week. At each assessment session, the clinical examinations like blood cell counts and routine biochemical tests were performed. Laboratory values were normalized by dividing their measurement by the upper limit of normal value used as a continuous variable. At time zero and week 2^{nd}, 4^{th}, 12^{th}, 24^{th}, 36^{th} and 48^{th} during the course of treatment, the HCV-RNA by RT-PCR was assessed. The presence of HCV-RNA in serum was checked at week 24 and 48 after the EOT during the period of follow-up.

2.6 Evaluation of biochemical parameters with reverence to the treatment

Histological and biochemical parameters were taken into consideration for the evaluation of treatment response in the patients. Various parameters which were considered were ALT, AST, ALP, WBC and platelet count. The traditional marker for assessing treatment response is normalization of the serum ALT level. Although this endpoint was established before identification of the HCV, it appears to be appropriate as measuring HCV-RNA for determining the initial response to interferon, i.e. normalization of ALT is usually associated with loss of detectable virus from the serum.

III. STATISTICAL ANALYSIS

All the data of the study were calculated as mean and standard deviation. Continuous and categorical variables were compared using Wilcoxon Mann Whitney U test and Fisher's exact test using the link http://ing.gsf.de/cgi-bin/hw/hwl.pl. The p value was calculated using the Student's t test and the statistical analyses were done using the statistic program link http://studentstest.com/?i=8%2F13&type=1&tails=2&tsubmit=calculate&j10%2F19. Microsoft Excel was used to generate the graphical data for SVR against HCV genotypes and subtypes. $P \leq 0.05\%$ was considered to be significant.

IV. RESULTS

4.1 *Demographic and clinical properties*

Out of total 50 enrolled subjects, 32 males and 18 female patients received treatment. The clinical profile of all the patients comprising of 50 patients with a mean age of 42.53, the route of HCV infection and the method of detection is summarized in table 1.

Table 1: Demographic and clinical features of the subjects enrolled in the study

Parameters	Value No. (%)
No. of patients	50
Age Y (Mean±SD)	42.53 ± 12.6
Sex (no. of patients/%)	
Female	18 [36%]
Male	32 [64%]
Route of infection (no. of patients/%)	
Accidental needle prick	4 [8%]
Unhygienic habits	6 [12%]
Blood transfusion	12 [24%]
Surgery	8 [16%]
Unsterilized Needles	8 [16%]
Unknown	12 [24%]
Mode of detection (no. of patients)	
ELISA	50
PCR	50

4.2 Virological response stratified by treatment group and genotype

The standard therapy (PEG-IFNα 2a and RBV) was given to all the patients and it was found that 6 out of 50 patients did not respond at all out of which 2 were males and 4 were females. We also observed that the non responders in case of genotype 1a were 3 (1 male and 2 females) and for genotype 1b were 2 (1 male and 1 female). In case of genotype 3a, there was only 1 non responder (female) and for genotype 3b there were no non responders. So we concluded that the response rate in case of genotype 3 (3a and 3b) was higher as compared to genotype 1 (1a and 1b) (**Table 2**).

Table 2: Virological response stratified by the treatment group against HCV subtypes

Genotype	EVR		SVR		NR		Total
	M	F	M	F	M	F	
1a	1	1	3	2	1	2	10
1b	1	0	5	3	1	1	11
3a	2	1	8	6	0	1	18
3b	2	2	6	1	0	0	11
Total	**6**	**4**	**22**	**12**	**2**	**4**	**50**

When analyzing the virological response in patients undergoing for the treatment were found that the patient's who continued to experience a sharp decline in viral load during the first 4-12 weeks of treatment had greater chance of achieving the SVR.

We found that 10 out of 50 patients showed EVR, out of which 6 were males and 4 were females, 2 patients who showed EVR in genotype 1a (one male and one female) and one patient who showed EVR in genotype 1b (one male). There were 3 patients under genotype 3a that showed EVR (2 males and 1 female) and 4 patients showed EVR in genotype 3b (2 males and 2 females). Hence patients with genotype 3 (3a and 3b) showed a better EVR as compared to the genotype 1(1a and 1b).34 out of 50 patients showed SVR out of which 22 were males and 12 were females. Under genotype 1a, 5 patients showed SVR (3 males and 2 females). Under genotype 1b, 8 patients showed SVR (5 males and 3 females). Under genotype 3a, 14 patients showed SVR (8 males and 6 females). Under genotype 3b, 7 patients showed SVR (6 males and 1 female). Hence, the SVR in case of genotype 3(3a and 3b) was more and in case of genotype 1(1a and 1b) was less. Hence, patients with genotype 3 (3a and 3b) have showed more SVR, better EVR and there were less non responders (NR) in them as compared to patients with genotype 1(1a and 1b) which showed less EVR, less SVR and more non responders (NR) (**Fig. 2a and 2b**).

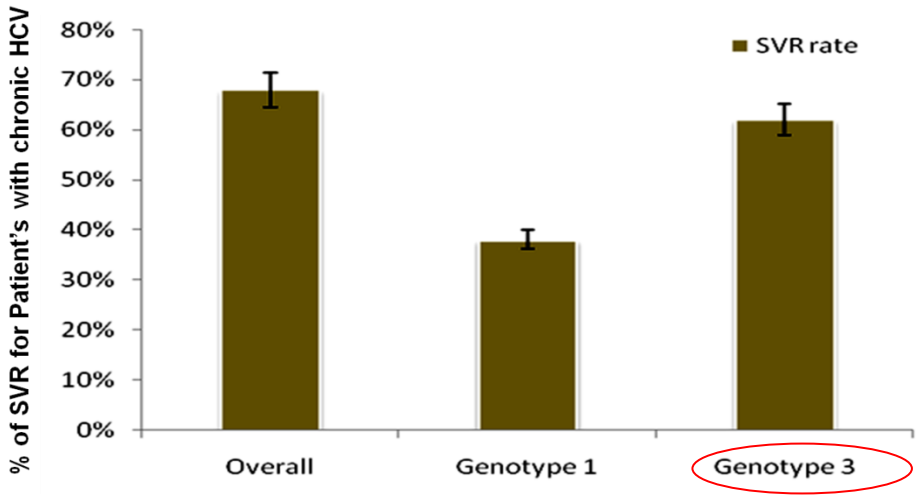

Fig. 2a: SVR rates in the total study population based on HCV genotypes

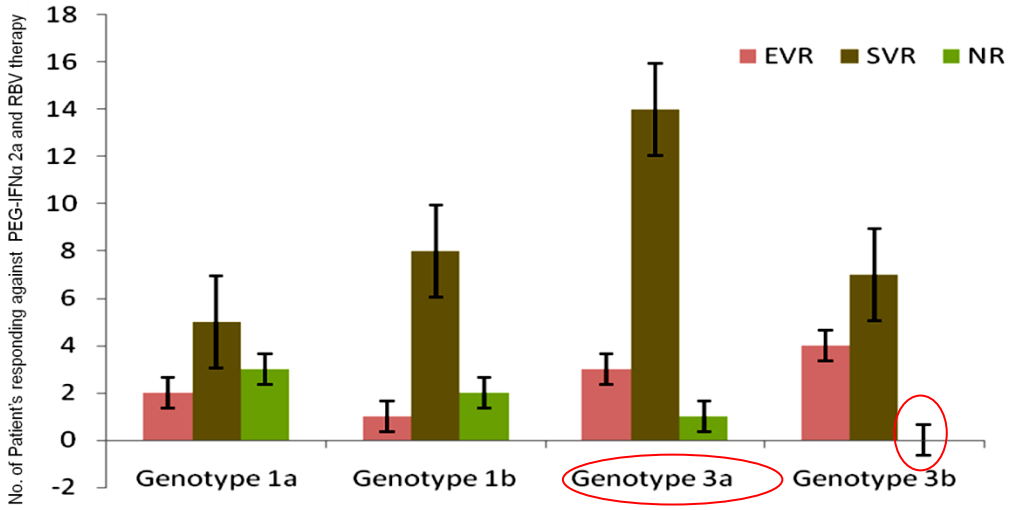

Fig. 2b: Graph showing the virological response stratified by treatment and genotype. With the genotype subtype on X-axis and no of patients with EVR, SVR and Non Responders on Y-axis

4.3 Predisposing factor associated with SVR

34 out of 50 (68%) patients treated with PEG-IFNα 2a and RBV achieving the SVR, neither gender, HCV subtype, pretreatment normalized AST, ALT, normalized hematological values nor therapy duration (24wk and 48wk) were found to be associated with treatment outcome. Only AST (p = 0.05%) was found significantly associated with SVR (**Table 3**)

Table 3: Mean and *p* value of overall patients with regard to their haematological parameters and serum enzymatic activities for positive and negative SVR against HCV genotypes

	Positive SVR (12/22)		Negative SVR (6/10)		*P* Value
	Genotype 1 (n=13)	Genotype 3 (n=21)	Genotype 1 (n= 8)	Genotype 3 (n=8)	
Gender (Female/Male)	(5/8)	(7/14)	(3/5)	(3/5)	0.30[a]
HCV Subtype (a/b)	(5/8)	(14/7)	(5/3)	(4/4)	0.96[a]
AST-N (mean ± SD)	56.7 ±0.74	40.76 ±0.04	44.8 ±0.27	39.5 ±0	0.05[b]
ALT-N (mean ± SD)	75.5 ±0.19	56.7 ±0.11	53 ±0	37.5 ±0	0.9[b]
ALP-N (mean ± SD)	107 ±0.12	97.6 ±3.97	107.1±0.15	122.5 ±0	0.91[b]
Hb-N (mean ± SD)	1.13 ± 0.12	1.15 ± 0.14	1.21 ± 0.09	1.34 ± 0.05	0.91[b]
Platelet x 10^3/µl (mean ± SD)	188 ± 51	192 ± 48	197 ± 54	201 ± 63	0.60[b]
WBC x 10^3/µl (mean ± SD)	5.98 ± 1.49	6.15 ± 1.53	6.87 ± 1.52	5.98 ± 1.61	0.06[b]
Therapy Duration/ regimen (weeks)					
24	5 (38.46%)	9 (42.85%)	3 (37.5%)	4 (50%)	
48	8 (61.53%)	12 (57.14%)	5 (62.5%)	4 (50%)	
IFN-α2a (dose)					
24 weeks (9-21 Bio/week)	0	0	2 (25%)	2 (25%)	
24 weeks (18-42 Bio/week)	3 (23.07%)	8 (38.09%)	1 (12.5%)	2 (25%)	
48 weeks (9-42 Bio/week)	8 (61.53%)	12 (57.14%)	4 (50%)	4 (50%)	
48 weeks (PEG-IFN-α 180 µg/week)	2 (15.38%)	1 (4.76%)	1 (12.5%)	0	

a= Fischer's Exact Test, b=Wilcoxon Mann- Whitney U Test

V. DISCUSSION

Virological response kinetics during therapy has emerged as important prognostic factors for the treatment of patients with chronic HCV infection. Absence of EVR at week 12 during therapy is the negative predator for non response to treatment. Patients with RVR defined as undetectable HCV RNA at week 4 of combination therapy have a high probability of achieving SVR. Conversely those without an RVR have considerably lower SVR rates and it is regarded as the most important predictor for SVR.The recommended treatment for patients with hepatitis C genotype 1 is PEG-IFNα 2a and RBV for 48 weeks. Such treatment has yielded overall SVR rates of 45%-55% in randomized control phase III clinical trials [21]. However, the treatment responses are not uniform across all populations and are dependent on various viral and host factors. In patients with genotype 1 higher SVR rates are obtained with 48 weeks than with 24 weeks treatment [20]. In the present study, we have observed that males had a better SVR and EVR as compared to females in both the genotypes (genotype 1 and genotype 3) and among the non responders there were less males as compared to females. It was seen that there were less females who showed EVR and SVR as compared to males. There were more females among the non responders as compared to males. Hence males had a better prognosis as compared to females under both the genotypes (genotype 1 and genotype 3).

VI. CONCLUSION

Our study with reference to previous studies have demonstrated that EVR, RVR, NR and most importantly SVR are important factors for the achievement of complete virological response against HCV genotypes and subtypes. The study also suggests that males have greater chance of achieving SVR as compared to the females in against the combination therapy for HCV genotype 1 and genotype 3.

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Conflict of interest: The authors declares that they don't have any conflict of interest

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