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Research Article

Pegylated Interferon (PEG-INF) Plus Ribavirin Response in Patients with HCV Genotype-3 in Hyderabad, Sindh

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Abstract

Objective

To determine the virological response after Pegylated interferon (PEG-INF) and Ribavirin therapy. To monitor the response Hepatitis C by HCV viral load RT-PCR in chronic HCV patients' genotype 3 in the different intervals of Rapid virologic response (RVR) and Sustained Virologic Response (SVR).

Material and Methods

All anti HCV antibody positive subjects, aged >18 years male and female were enrolled in the study from the Gastroenterology department Asian Institute of Medical Sciences (AIMS) Hyderabad and Gastro-wards at Isra University Hospital Hala road Hyderabad. Quantification of HCV RNA was done by the techniques of target amplification. HCV genotype was done by Abbott Real Time HCV Genotype II assay "Abbott Molecular, USA". The patients were treated with combination of Pegylated interferon (PEG-INF) and Ribavirin, in the course of 4 weeks treatment to achieve the RVR and further 24 weeks to achieve the SVR. All the data was recorded in the proforma.

Results

Total 200 patients were includes males 54% and females 46% with mean age of 36.0±12.0 years. Majority of patients 114(57.0%) exhibited level of viral load >500000IU/mL, while 86(43.0%) patients revealed <500000IU/ml. RVR was achieved by 61% of the patients, while (SVR) was achieved by 83% of the patients following by the 4 weeks and 24 weeks of therapy respectively. Viral load >500000IU/ml showed significant impact on RVR p-value 0.009.SVR was achieved within 83%, particularly as 25.6% having viral load >500000IU/ml and 30.7% having viral load <500000IU/ml, on the basis of these findings higher viral load had negative significant impact on viral load achievement p-value 0.003.

Conclusion

It is concluded that Pegylated Interferon (PEG-INF) plus Ribavirin has tremendous response in patients with HCV genotype-3.Viral load >500000IU/ml and <500000IU/ml showed were significant impact.

Introduction

Hepatitis C virus (HCV) is a worldwide public health challenge, projected that 71,000,000 individuals are infected with chronic Hepatitis C virus (CHCV). Infection having significant risk of acquiring advanced hepatic disease, as cirrhosis and carcinoma [1]. An increased risk of hepatocellular carcinoma (HCC) by 17-times in chronic hepatitis C patients compared to their corresponding individuals, who are HCV-negative and this can possibly persist even after establishing a therapy-provoked sustained virological response (SVR) [2-5]. Untreated around 399 000 individuals died of consequences correlated with HCV, predominantly of advanced hepatic disease and HCC, yearly [6-9]. As a concern, Hepatitis C is the commonest indication for hepatic transplantation within developed nations [10]. For above 20 years, interferon has been a source for the treatment of HCV, responses was enhanced during 1998 via Ribavirin addition and then during 2001-2002 via joining the interferon molecule to polyethylene glycol (PEG) [11-13]. In recent time major advances have been made in the treatment of hepatitis C with licensing of 1st direct acting antivirals and several proceeding trials with different direct acting antiviral sex habiting advantageous tolerability profile,

high potency, decreases induration of treatment higher reduction to resistance and all oral procedures [14]. Though, yet no vaccine is accessible.

HCV exposed during 1989[15], as a single stranded RNA virus of the Flavivaridae family, which as well includes several human pathogens of Flavivirus genus that are transmitted by arthropods such as yellow dengue virus, West Nile virus and fever virus. Isolates of HCV have been categorized into 7 genotypes and several subtypes [16] having distinct geographical allocations and sensitivity to treatment based on interferon [17-18]. Pakistan is an under developed nation of 170,000,000 individuals with low educational and health standards. Within Pakistan 10,000,000 individuals are assumed to be contaminated by HCV [19]. Public wellbeing authorities are establishing awareness regarding hepatitis via electronic and print media [20]. However, still remarkable endeavors are needed to elevate the consciousness of several risk factors concerning the transmission of HCV. The WHO has instituted an international standard to universally standardize HCV RNA quantification unit's defined international unit currently applied in every commercial quantitative assays of HCV RNA. In "real-time" PCR, every round of amplification results in a fluorescent signal emission and total signals per cycle are proportional to HCV RNA quantity in the starting specimen. Assays of qualitative detection defined the presence of HCV either present or not in blood stream. We aimed in current study, to assess the efficacy of Pegylated interferon plus Ribavirin (PEG-INF) treatment monitoring via HCV viral load by RT-PCR among CHCV patients' genotype 3 via molecular analysis in the course of Ribavirin plus Pegylated interferon treatment in term of RVR and SVR.

Materials and Methods

The study was conduct at Institute of Biochemistry, University of Sindh. All anti HCV antibody positive subjects, aged above 18 years with both male and female genders were enrolled in the study from the gastroenterology department Asian Institute of Medical Sciences(AIMS) Hyderabad and gastro-wards at Isra University Hospital Hala road Hyderabad. The HCV RNA positive patients (n=200) were considered for genotyping. Samples of Venus blood were aseptically collected for genotyping in 2 yellow-top gel-tubes. 3ml of blood was isolatedin the course of one hour and then stored at -70C to be used as one-time batchanalysis.

Target Amplification and RNA Extraction

Viral RNA was isolated from the plasma of patient's by QIAamp Viral RNA mini kit as per the instructions of manufacturer. A positive sample (positive extraction) and two negative samples (negative extraction) were taken in every batch for assessing the efficiency of extractions.

HCV RNA Quantification

Quantification of HCV RNA was done by the techniques of target amplification and the Artus HCV kit amplifies a 240 bp region of 5 UTR HCV genome with the detection directly to specific amplicon in Rotor-Gene Q fluorescence channel Cycling Green. Heterologous internal control is as well amplified for checking potential PCR inhibition and it is detected in the fluorescence channel Cycling Orange of the Rotor-Gene Q. For detection of Artus HCV QS-RGQ, lower limit is 36.2IU/ml, whereas the linear range is 67.6IU/ml. The assay obtained in recent times the Conformity European- *in Vitro* "Diagnostic Medical Devices approval".

HCV Genotyping

Determination of HCV genotype was done by Abbott RealTime HCV Genotype II assay "Abbott Molecular, USA". This assay targets 5-UTR region that is higher preserved in HCV genotypes, and NS5B gene for efficient distinction between HCV subtypes 1b and 1a.The assay employs three reaction blends with HCV oligo - nucleotide probes bound to three distinct reporter dyes (NED, VIC, and FAM) and a 4th dye (Quasar 670) allocated to pumpkin gene, a heterologous internal control. This pattern lets a precise distinction of HCV type's I–VI and subtypes 1a, 1b "Ciotti *et al.,* 2010".

Statistical Analysis

The Analysis of data was done by SPSS-22.0 (SPSS Inc, Chicago, US). Descriptive statistics was applied for summarizing the categorical and continuous data. Results were exhibited as percentages, frequencies, and mean ± standard deviation.

Results

The demographic, virological and clinical features of the patients are enlisted in Table 1. The regimens of treatment were relatively heterogeneous, even though the patients were managed with a combination of Ribavirin and Peg-IFN therapy. Total 200 patients were treated in the course of 4 weeks (RVR) and finally 24 weeks (SVR). The gender distribution exhibited male 54% and female 46%. At the beginning of treatment, every patient was positive for both of the parameters. Mean of ATL was 61.23±45.22IU/L. The HCV-RNA baseline values varied from >500000IU/ml and <500000IU/ml. The levels distribution exhibited that majority of patients (114/200; 57.0%) exhibited levels above 500000IU/mL, while 86 of the 200 patients (43.0%) revealed values below 500000IU/ml. Moreover, there were no significant differences between male and female in whole study population. [Table.1A &B]

Table 1A: *Baseline Characteristics Patients, n=200*

Characteristics	Value
Male	108 (54.0%)
Female	92 (46.0%)
Age, Years	(36.0±12.0)
ALT IU/L	(61.23±45.22)
Low Viral Load<500000IU/ML	86 (43%)
High Viral Load>500000IU/ML	114 (57%)

Table 1B: *Sustained virological response of HCV patient's genotype 3 by gender wise: n=200*

Sustained Virological Response	Respondent	Non-respondent	P -Value	Total
Male	73	35	0.316	108
Female	66	26		92
Total	139	61		200

As expected, the reduction in HCV-RNA in the course of therapy was much rapid since the negativity rates for HCV-RNA, and (RVR) achieved by 61% of the patients, while (SVR) was achieved by 83% of the patients following by the 4 weeks and 24 weeks of therapy respectively. Subjects having lower HCV viral showed good response to treatment as compare those having HCV viral load above 500000 IU/ml. [Fig.1] Subjects having lower HCV viral showed good response to treatment as compare those having HCV viral load above 500000 IU/ml.

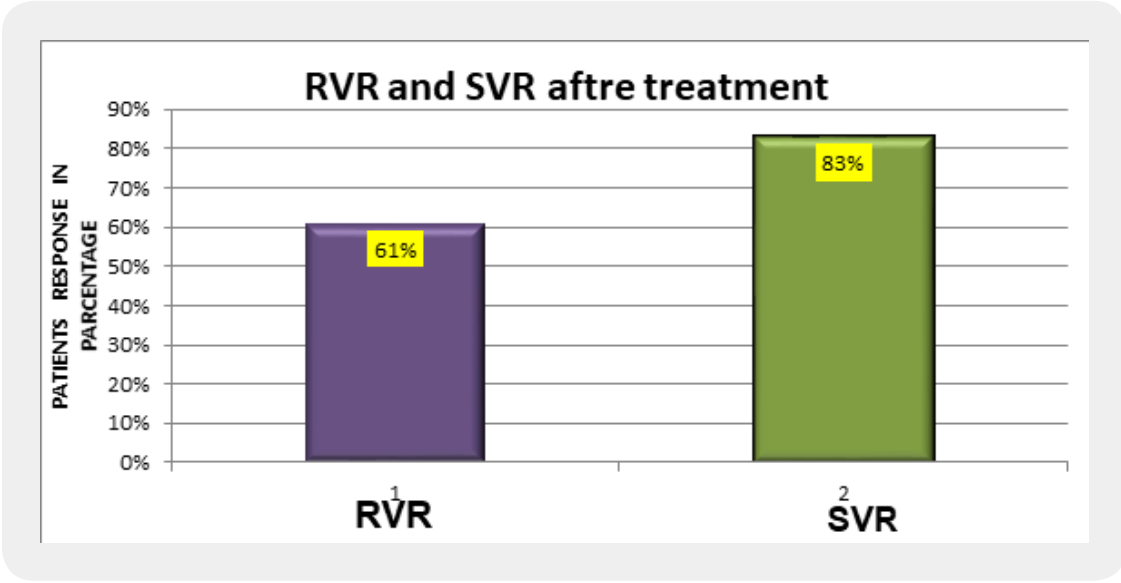

Figure 1: *Pegylated interferon response in RVR and SVR N=200*

Viral load >500000IU/ml showed significant impact on RVR, as majority of the 61 showed RVR out of 86 cases presented with viral load <500000IU/ml, while 61 cases showed RVR out of 114 those had viral load >500000IU/ml, p-value 0.009 [Table:2]

Table 2: *Impact of viral load >500000IU/ml and <500000IU/ml on (rapid virological response)n=200*

HCV Viral Load	Responders	Non-responders	*P=Value*
>500000IU/ml	61(30.5%)	53(26.5%)	0.009
<500000IU/ml	61(30.5%)	25(12.5%)	
Total	122(61%)	78(39%)	

SVR was achieved within 83% out of the 200 cases, particularly as 25.6% having viral load >500000IU/ml and 30.7% having viral load <500000IU/ml, on the basis of these findings higher viral load had negative significant impact on viral load achievement p-value 0.003. [Table:3]

Table 3: *Sustained virological response impact of viral load >500000IU/ml and <500000IU/ml on HCV genotype 3 patients n=200*

HCV Viral Load	Responders	Non-responders	*P=Value*
>500000IU/ml	87(25.6%)	27(34.6%)	.003
<500000IU/ml	79(30.7%)	7(8.9%)	
Total	166(83%)	34(17%)	

Discussion

Incidence of Hepatitis C patients is high globally and rapidly elevating in Pakistan. Compulsory screening for blood donors and growing screening trend for the patient's prior to dental and surgical procedures as well unawareness of diseases is contributing significantly in uncovering fresh cases of Hepatitis C. Genotype 3 was dominant in this series. In this study males were 54% and 46% females. While in the study of Sandoughdaran S *et al* [21] reported that males were 81.1% and females 19.9% these are in the favour in our findings but a big difference in gender as compare to this study.

Genotype 3 is a poorly understood and the most underrated type of HCV. For years, we considered genotype-3 as good with favourable interferon therapy outcome. However, most Asian studies have exhibited lower SVR contrasted to western data, Shahid Sarwar *et al* [22] noted 72.1%, Batool *et al* [23] noted 68% and Waheed yaser *et al* [24] observed 58%-75% response rate in genotype 3 while some higher response was also occurred Iqbal S *et al* [25] showed SVR 89.23% and ETR 81.5% HCV genotype 3patients.However, it was 72% in a study from Lahore consisting of 721 cases [26]. Niederau C *et al*. established that genotype 3 patients have further hepatic fibrosis and just 56.9% of them were responsive to interferon treatment [27]. In our study, SVR of 72.1% patients supports the other documented studies [28]. It is distressing to perceive that fresh evolving directly acting drugs with assurance of interferon free therapy of HCV are documented to be not much effective in genotype 3 [29]. Effect of genotype-III on the resistance of insulin and the metabolism of lipids is assumed to be a factor accountable for poor treatment response of it [30].

To address this problem of insufficient response, response directed treatment was introduced. RVR, is directly correlated with viral clearance [31], also noticed in present study. In present study SVR in HCV genotype 3 patients was 56.4% and overall SVR response 83% and non-responders were 17% while RVR was 61% statistically significant. It has been reported earlier Fried MW *et al* noticed that subjects with RVR are superior SVR between 88 and100% across all genotypes [32], however SVR in cases without RVR is lower, 45% documented by Shiffman ML [33], and 56% in the study of Dalgard O [34], with 24 week therapy. These results confirmed the protective role of Pegylated interferon response because of small number of HCV genotype 3 patients. In the latest study November 2016 on 288Myanmar HCV patients genotype 1,2,3, and 6 treated with Peg-IFN alpha 2a-2b and RBV observed SVR 82% overall in population of Myanmar [35] has been similar as present study associated with the protective role and brilliant response of therapy in HCV patients.

We investigated significantly achieved RVR and SVR (61%), *P=.009* and 166(83%), *P= .003*) during Peg IFN and RBV therapy. This is in agreement of previous study described HCV with thalassemic patients achieved (72.2% and 77.8% having a only significant factor that correlated with SVR in thalassemic cases. Our study gives rise to the challenges of acceptability and compliance of importance of therapy among cases. Only 17% of cases, requiring extended treatment, continued therapy beyond 6 months. Regardless of, approving RGT plan at the treatment initiation, most patients denied treatment extension because of therapy associated side effects.

Response directed interferon treatment can marginally improve the interferon treatment response as noted in this study, however because of insignificant compliance of the prolonged treatment, number of cases on Peg-IFN and RBV were limited, there is needed of big case control studies to establish better outcomes. As prohibitively higher price of DAAs, and non-affordable for the most of our populace; it is thus, prudent to additionally explore another option Peg IFN and RBV to treat cases with HCV in Pakistan.

Conclusion

It is concluded that Pegylated Interferon (PEG-INF) plus Ribavirin has excellent response in patients with HCV genotype-3. Viral load >500000IU/ml and <500000IU/ml showed significant impact on RVR and SVR as compare to. No significance has achieved in male and female patients. Treatment planning should be upgrade to achieve the RVR and SVR in patients with higher viral load.

Conflict of Interest: None

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