ORIGINAL ARTICLE

Pre-treatment Predictors of Response for Assessing Outcomes to Standard Treatment in Infection with HCV Genotype 3

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ABSTRACT

Objective: To determine the role of pre-treatment predictors of response in assessing outcomes to standard treatment in HCV genotype 3.

Study Design: Observational study.

Place and Duration of Study: Department of Medicine, KRL General Hospital, Islamabad, from December 2004 to December 2006.

Methodology: All patients with positive anti-HCV and PCR genotype 3a were recruited and written and informed consent was taken. Patients were treated with standard Interferon plus Ribavirin therapy (IFN alpha-2a, 3MU t.i.w 24 weeks plus Ribavirin 1000-1200 mg/day) for 6 months. The effect of pre-treatment factors influencing outcome i.e. age, gender, weight, baseline ALT, necroinflammatory grade, fibrosis and steatosis on the final outcome were further analyzed by univariate logistic regression analysis.

Results: Response rates to standard Interferon plus Ribazole therapy were studied in 190 patients. The end-of-treatment complete response (EOTCR) was seen in 81% (n=155) of the patients, whereas 17% (n=33) were non-responders (NR). Sustained viral response (SVR) was seen in 58% (n=112) patients and 24% (n=45) were relapsers. SVR was higher in patients without steatosis (OR = 2.52, 95% CI = 1.356- 4.71 , p = 0.04). Higher SVR was seen in patients weighing less than 65 kg, as compared with weight > 65 kg (OR= 2.277, 95% CI = 1.246- 4.161, p = 0.007). The other variables were not found to be significantly associated with improved SVRs.

Conclusion: Out of the studied predictors, body weight and presence of steatosis, were statistically related to treatment outcome. Pre-treatment host factors can predict response to treatment that can help in individualizing treatment and patient selection and optimize treatment outcomes.

Key words: *Chronic hepatitis C. Predictors. Interferon. Ribavirin. Steatosis. Body weight. Sustained viral response.*

INTRODUCTION

Chronic hepatitis C virus infection affects 130-170 million or approximately 2.2-3% of world's population.[1] It is estimated that approximately 10 million people are infected with HCV in Pakistan with an average prevalence rate of 6%.[2,3] Type 3 is the most prominent genotype in Pakistan with a prevalence of 75-90%.[4]

Response rates to treatment in genotype 3 infection are not as good as previously believed resulting in an increasing need to optimize outcomes. Various pre and on treatment factors (both host and viral) have been studied for their predictive power on the outcomes and response to therapy. Pre-treatment factors (host baseline factors) such as age, gender, ALT levels and extent of liver disease are considered to be weak predictors of response to interferon-based therapy.[1-9] Viral factors including genotype, viral load and mode of acquisition of infection are considered more reliable pre-treatment indicators of response.[3,5,9,10] However these have been studied mostly in Western cohorts, and with combined genotype 2 and 3 infection.

This study assesses the predictive power of pre-treatment host predictors of response in genotype 3 patients treated with standard interferon and ribavirin, to facilitate patient selection and help the physician in individualizing treatment regimens and optimize treatment outcomes.

The objective was to to determine the role of pre-treatment predictors of response in assessing outcomes to standard treatment in HCV genotype 3.

METHODOLOGY

This observational study was carried out at the Medical Department of KRL General Hospital, Islamabad from December 2004 till December 2006. All patients provided written informed consent.

Eligible patients were previously untreated adults who had HCV RNA detectable in serum by PCR with genotype 3a; who had undergone liver biopsy within one year before entry that was consistent with chronic hepatitis, and who had ALT values from normal (> 30 IU/L for men

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and 19 IU/L for women) to four times the normal, with the hematological and biochemical values of hemoglobin; white blood count, platelet counts, bilirubin, albumin, prothrombin time and creatinine within normal limits. Patients were excluded if they had decompensated cirrhosis; other causes of liver disease, seizure disorders, cardiovascular disease, hemoglobinpathies, thyroid disease, hemophilia, poorly controlled Diabetes, autoimmune disease, previous organ transplant or if they were unable to use contraception.

All the patients fulfilling the inclusion criteria were treated with interferon 2b alpha 3mu sub-cutaneously three times per week, plus ribazole 1000-1200 mg/day. The dose of ribavarin was adjusted according to the body weight (1000 mg for weight below 75 kg and 1200 mg for weight 75 kg or more). Treatments were administered for 24 weeks with a subsequent 24-week follow-up period. During treatment patients were assessed as outpatients at weeks 2, 4, 8, 12, 16, 20 and at 24 , and then at 24 weeks after the end of the therapy. Qualitative PCR for HCV RNA was done at weeks 0, 4, 24 and 48. At each visit, blood cell counts and ALT were measured and recorded. Side effects were also recorded at each visit and were graded as mild, moderate, severe and life threatening.

A sustained virological response (SVR) was defined as undectable HCV RNA by a qualitative PCR test 6 months after stopping treatment in patients who had achieved end of treatment responses. An EOTCR i.e. end of treatment complete response was defined as undectable HCV RNA by qualitative PCR at 24 weeks of the treatment. Non-response was defined as a positive qualitative PCR at any time before or at 24 weeks of the treatment. Relapse was defined as a positive PCR between 24-48 weeks in those who had a negative PCR at 24 weeks.

All PCR tests were done by a Cobas Amplicor with a lower cut off value of 100 copies/ml.

The variables studied were age, gender, weight, positive family history, baseline ALT and liver histology.

All patients underwent a trucut liver biopsy. Biopsy specimens were classified by the HAI Knodell Score. Necro inflammatory grade was classified as mild (score 1-4), moderate (5-10), and severe (5-10). Fibrosis was classified as F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis or bridging necrosis) and F4 (cirrhosis). Steatosis was categorized by presence or absence.

The distribution of individual characteristics was evaluated by simple descriptive statistics. To compare the overall distribution of response, end of treatment complete response, non-responder, relapse, sustained response and its association with different variables, the electronic database organized in SPSS for windows version 15 was used. Quantitative data i.e. age, height, weight and ALT was presented as mean ± SD.

Frequencies and percentages were calculated for qualitative data i.e. gender, family history, weight (group 1 : < 65 kg , group 2: > 65 kg), ALT (normal, between 2-4 times normal and > 4 times normal), findings on liver biopsy i.e. activity (mild, moderate, marked), fibrosis (stage 1-2 and 3-4) and steatosis (present or absent). To see the effect of these variables on sustained virological response, univariate logistic regression was applied. P-value less than 0.05 was considered significant.

Sample size was calculated by using WHO sample size calculator (sample size determination in health studies, a practical manual, software version by KC Lun and Peter Chiam, National University of Singapore) taking confidence level of 95%, anticipated population proportion 77.5%.[9,10] (proportion of patients who achieved SVR) and relative precision 8%. Sample size was equal to 175.

RESULTS

Response rates to standard interferon plus ribazole therapy were studied over a 2 years period. Out of a total cohort of 250 patients 60 were excluded; 30 patients did not meet inclusion criteria, 23 were lost to follow-up and 7 declined treatment.

A total of 190 patients were evaluated for the influence of potentially important factors on SVR. The mean age of patients was 39.79±8.13 years and mean weight was 66±9.5 kg. The mean value of alanine aminotransferase was 98±68.8 IU/L with a range of 14-496 IU/L. The mean platelets count was 199677±59068 mm^3 with a range of 48000-331000 mm^3. Family history was positive in 19.6% of patients. The main risk factors were a history of transfusions in 11%, prior surgeries in 25%, dental procedures in 20%, and multiple parenteral injections in 8%, 34% of patients did not have any history of exposure to known risk factors. The histology at liver biopsy showed mild necroinflammatory activity in 39.5% of patients, moderate activity in 48.5% and marked activity in 22% of patients.

In the total of 190 patients, end of treatment complete response (EOTCR) was seen in 81% (n=155), whereas 17% (n=33) were non-responders (NR). Sustained viral response was seen in 58% (n=112) patients, 24% (n=45) were relapsers giving a relapse/non-response rate of 41%.

To examine the influence of potentially important prognostic factors on SVR, factors known to affect response (HCV genotype, cirrhosis, age, gender, and baseline weight, activity at liver biopsy, fibrosis and steatosis) were examined. The influences of these pre-treatment host factors on SVR were examined individually by univariate logistic regression analysis on

Table I: Predicatability of demographic characteristics in achieving SVR.

Prediction variables	SVR n=112 (58.9%)	NR/relapser n=78 (41.1%)	OR (95% CI)	p-value
Age of patients				
Group 1 = 20 - 44 years	n = 48 (53.3%)	n = 42 (46.7%)	1.556 (0.87-2.783)	0.137
Group 2 = 45 - 65 years	n = 64 (64%)	n = 36 (36%)		
Gender				
Male - 49.5% (n = 94)	n = 56 (59.6%)	n = 38 (40.4%)	1.053 (0.59-1.877)	0.862
Female - 50.5% (n= 96)	n = 56 (58.3%)	n = 40 (41.7 %)		
Positive family history				
Negative - 80.5% (n=153)	n=91 (59.5%)	n= 62 (40.5%)	1.118 (0.541-2.311)	0.763
Positive - 19.5% (n=37)	n= 21 (56.8%)	n=16 (43.2%)		
Body weight				
Group 1 = < 65 kg	n=58 (51.8%)	n=25 (32.1%)	2.277 (1.246-4.161)	0.007
Group 2 = > 65 kg	n=54 (48.2%)	n=53 (67.9%)		

Table II: Predicatability of laboratory parameters in achieving SVR.

Prediction variables	SVR n=112 (58.9%)	NR/relapser n=78 (41.1%)	OR (95% CI)	p-value
Baseline ALT levels				
Normal - n=29 (15.3%)	n= 22 (75.9%)	n= 7 (24.1%)	–	0.087
Between 2-4 times normal - n=128 (67.4%)	n=36 (53.9%)	n= 59 (46.1%)	2.687 (1.072-6.735)	0.035
ALT > 4 times normal n=33 (17.4%)	n= 21 (63.6%)	n= 12 (36.4%)	1.796 (0.593-5.436)	0.3
Liver biopsy				
Mild activity - 39.5% (n=75)	n= 49 (65.3%)	n=26 (34.7%)	–	0.172
Moderate activity - 48.4% (n=92)	n=53 (57.6%)	n=39 (42.4%)	1.387 (0.738-2.604)	0.309
Marked activity - 12.1% (n=23)	n=10 (43.5%)	n=13 (56.5%)	2.45 (0.946-6.346)	0.065
Fibrosis				
Stage 1-2 = 96.8% (184)	n=110 (59.8%)	n=74 (40.2%)	2.973 (0.531-16.646)	0.215
Stage 3-4 = 3.2% (n=6)	n=2 (33.3%)	n=4 (66.6%)	–	
Steatosis				
Yes - 60.5% (n=115)	n=58 (50.4%)	n=57 (49.6%)	2.527 (1.356-4.71)	0.004
No - 39.5% (n=75)	n=54 (72.0%)	n=21 (28.0%)	–	

each factor for the outcome of SVR and are shown in Table I and II.

The effect of demographic characteristics on achieving sustained virological response is given in Table I which shows higher SVR rates of 64% in older age group (45-65 years) versus 53% in age group less than 44 years (OR=1.556, 95%CI=0.87-2.783, p=0.137), showing a 1.55 times greater chance of achieving SVR in those in the older age group but this difference was not statistically significant. Response rates were similar in both genders 59.6% in males versus 58.3% in females. (OR=1.053, 95%CI=0.59-1.877, p=0.862). There was no association of a positive family history with achieving SVR (OR=1.118, 95% CI=0.541-2.311, p=0.763) as given in Table I.

The SVR rate was significantly related with weight, with those weighing less than 65 kg having a 2.277 times more chances of achieving SVR as compared to those in the higher weight category (OR=2.277, 95%CI=1.246-4.161, p=0.007). This difference was statistically significant (Table I).

In patients with normal ALT at baseline, 75.9% achieved SVR compared to 53.9% SVR rates in those with ALT between 2-4 times normal and 63.6% SVR rates in those with ALT greater than four times normal. There was a 2.68 times greater chance of achieving SVR in patients with twice to four times normal ALT level (OR=2.687, 95% CI=1.072-6.735, p=0.035) as compared to patients with normal ALT levels. Similarly patients having ALT level more than four times normal also had 1.796 times greater chances of achieving SVR (OR=1.796, 95% CI=0.593-5.436, p=0.30) as compared to the normal ALT level patients, but this was not statistically significant (Table II).

In patients achieving SVR, liver histology showed mild necroinflammatory activity in 65.3%, moderate activity in 57.6% and only 43.5% patients with marked activity achieved SVR. However, there was a 2.45 times more chances of achieving SVR in patients with marked activity (OR=2.45, 95% CI=0.946-6.346, p=0.065) compared to those with moderate activity and a 1.38 times chance of achieving SVR(OR=1.387, 95% CI=0.738-2.604, p=0.309) as compared with mild activity. Both these differences were statistically insignificant. SVR of 60% was seen in patients with baseline fibrosis stage 1-2, versus SVR of 33% in those with fibrosis stages 3 and 4. Patients with lower stage of fibrosis (stage 1-2) had a 2.973 times more chances of achieving SVR as compared with patients of fibrosis stage 3-4 (OR=2.973, 95% CI=0.531-16.646, p-value=0.215) (Table II).

Steatosis was seen in 60% of liver biopsies; 50% of patients with steatosis achieved SVR versus SVR rates of 72% in those without steatosis. There was a 2.52

times increased chance of achieving SVR in patients without steatosis (OR=2.527, 95% CI=1.356-4.71, p=0.004) as compared` to those with steatosis.

DISCUSSION

Interferon/peg interferon in combination with ribavarin is currently the only effective treatment modality available for the treatment of chronic hepatitis C.[2,4,7,9,10,12] The initial estimate of over 80% response rates with combination therapy in genotype 3 appears to have been optimistic.[15,16] The response rate in this study was 65% versus over 80% in previous trials. This is consistent with author's previous data,[16] and recent international studies;[17-19] wherein previously reported rates of over 80% were shown to be due to combining genotype 2 with its higher response rates with those of genotype 3.

This has led to renewed interest in identifying pre-treatment patient characteristics associated with a greater or lesser likelihood of response to interferon. Pre-treatment characteristics including younger age, female gender, low body mass, low pre-treatment HCV RNA level, loss of detectable HCV RNA during the initial month of treatment, non-type 1 viral genotype, absence of fibrosis or cirrhosis, higher or longer doses of interferon, and low serum ferritin or hepatic iron levels have been associated with a greater likelihood of response to interferon. Yet none of these have been able to accurately and consistently predict the patients who will respond to interferon.

Genotype has been considered to be the most important virological factor in determining the response to treatment. HCV genotype 3 has shown better response rates both with standard and pegylated interferon as compared to genotype-1[3-5] but has lower rates compared to genotype 2. All patients in this study had genotype 3.

A low response to treatment is generally associated with male gender; females are considered to be better responders to interferon based treatments.[2] It was not observed in this study. There were an equal number of males and females, with slightly lower response rates seen in females but the difference was not statistically significant.

Age is also considered to be a weak predictor of response.[2,6] Age below 40 years is associated with better response, again not seen in this study. Most of the sustained responders were between 45-60 years of age. However, the differences were not statistically significant.

A low baseline body weight is predictor of a sustained virological response and increased body weight has been shown to be associated with low sustained virological response in genotypes 1, 2 and 3.[6-8] This was also found to be statistically significant in this cohort of patients as higher SVR rates of 52%, were seen in those with a lower body weight, (< 65 kg), compared to a lower SVR rate of 48% in those with a higher weight of > 65 kg. Most of the non-responders and relapsers were found to be overweight. Dietary counselling prior to initiation of therapy may improve therapeutic response.

Individuals with HCV may have normal ALT values and patients with HCV and normal ALT are more likely to be women. This was also seen in this cohort as well.

Liver biopsy provides an estimation of prognosis as well as an indicator of the likelihood of response to treatment. Patients with histologically mild to moderate liver injury as graded by HAI Knodell Scoring respond better as compared to those with marked activity. Most of the patients who showed sustained viral response had moderate activity. This observation was also not found to be statistically significant. Patients with compensated cirrhosis were excluded in this cohort.

Hepatic steatosis is a common histological feature of chronic hepatitis C. Steatosis has been reported to be more common in patients with genotype 3 infections and its severity is directly related to degree of necro-inflammatory changes.[11-13] Other factors like obesity, high alcohol consumption, type 2 Diabetes and hyper-lipidemia can also be associated with hepatic steatosis. Whatever the treatment regimen, presence of moderate to severe steatosis at pre-treatment liver biopsy is a highly significant predictor of failure to achieve SVR independent of other factors.

A significant inverse correlation exists between hepatic iron stores and the response rates.[14] Elevated serum iron markers are associated with male gender, alcohol consumption and increased liver inflammation and fibrosis. This factor was not studied in this cohort of patients but it should also be considered.

However, the accuracy of other pre-treatment host factors in predicting response is poor and precludes their use in clinical selection strategies. Investigators will need to identify new factors associated with response to treatment regimens and test whether algorithms, that include multiple factors can be developed to identify more accurately, those who would benefit from treatment.

CONCLUSION

The response rates to standard interferon and ribavirin treatment were lower than previously believed. Assessment of pre-treatment predictors of response, which have a significant effect like weight and steatosis may help to optimize outcomes and decrease an ever expanding pool of non-responders and relapsers.

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