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Treatment Induced Decline in Hematological Cell Lines as a Predictor of Response to Treatment with Conventional Interferon plus Ribavirin for Chronic Hepatitis C Infection in Pakistan

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Abstract: Background and aims: To determine the value of treatment induced decline in hematological cell lines as a predictor of response to conventional interferon combination therapy for chronic hepatitis C infection in Pakistan. The study was conducted by recruiting 129 treatment naïve individuals with chronic hepatitis C infection and treating them with conventional interferon plus ribavirin for 6 months. 115 individuals completed the study and were thus included. All subjects were analyzed for decline in hematological cell lines as predictors of response to treatment. Chi-square test was used for univariate analysis. Multivariate analysis was conducted using forward stepwise logistic regression. Male individuals constituted 31.3 % (n=36) of the population while 68.7% (n=79) were females. Mean age was 44.18 ± 8.81 years. Out of the total population, 30.4% (n=35) individuals developed anemia, 32.2% (n=37) experienced thrombocytopenia while leukopenia was seen in 50.4% (n=58). End of Treatment Response (ETR) was achieved by 80.9% (n=93) while Sustained Virological Response (SVR) by 58.3% (n=67). Leukopenia was associated with a better response rate i-e-, 69% (n=40) on univariate analysis (p=0.03*) but was insignificant on multivariate analysis (p=0.28). Anemia and thrombocytopenia were both insignificant predictors of SVR and ETR (p>0.05). It was thus concluded that decline in hematological cell lines during treatment of HCV genotype 3 in Asians using conventional interferon combination therapy is not associated with response rate. Leukopenia however, may have some effect on the outcome as an independent predictor.

Key words: Conventional Interferon · Anemia · Leukopenia · Thrombocytopenia

INTRODUCTION

Hepatitis C is a slowly progressive disease of the liver that is caused by infection with hepatitis C virus (HCV). Some people infected with hepatitis C spontaneously clear the virus but up to 85% of exposed people develop chronic hepatitis. About 20-30% of those initially infected develop cirrhosis within 20 years and a small percentage of these are at high risk of hepatocellular carcinoma [1,2]. The standard of care for chronic hepatitis C virus has improved considerably since the approval of interferon therapy. However despite improvements in treatment, many patients still do not respond adequately to initial therapy [3].

In order to predict the response to treatment with interferon, various parameters including clinical, pathological, histological, viral and host factors have been used. Among these, the role of treatment induced cytopenias is a topic of active debate. Anemia has been proposed both to be a positive as well as an insignificant predictor of Sustained Virological Response (SVR) [4,5]. Similarly, the significance of leukopenia and thrombocytopenia as independent factors for predicting response to interferon is controversial [5- 7].

The objective of our study was to determine the significance of decline in hematological parameters as a predictor of response to conventional interferon plus ribavirin therapy for chronic HCV. This is because

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accurately predicting the patients who will respond to therapy is becoming increasingly important, both from the point of patient care as well as health care cost. It will help to guide clinicians as they need to continue treatment in patients who will respond and stop treatment in patients who are unlikely to respond. Further studies on the factors that predict treatment response and tailoring the treatment based on these is required if we aim to conquer this disease.

MATERIALS AND METHODS

Ethics: This study was approved by the Ethical Review Committee for Research, KRL Hospital Islamabad. All patients provided informed written consent. All the work is in accordance with the ethical standards of the responsible committee on human experimentation and with the latest (2008) version of Helsinki Declaration of 1975.

Inclusion and Exclusion Criteria: Interferon naïve adults of both gender and all ages seen at liver clinic, KRL hospital with chronic Hepatitis C genotype-3 infection were eligible for enrolment and included in the study. Patients were required to have a detectable serum HCV RNA (ribonucleic acid) on Polymerase Chain Reaction (PCR) at presentation. There were also required to have a negative pregnancy test and having minimum values for hemoglobin of 120 g/l for women and 135 g/l for men; leukocyte count = 4x109/l and platelet count = 150 x 109/l initially. It was also required that they have normal bilirubin, albumin, urea and creatinine levels. Patients were excluded if they had decompensated cirrhosis, other causes of liver disease and/or were Hepatitis B surface antigen or Human Immunodeficiency Virus positive. Alcoholics, patients with seizure disorders, cardiovascular disease, hemoglobinopathies, thyroid disease, clinically relevant depression or any other psychiatric disease were also excluded. Other exclusion criteria included hemophilia, poorly controlled diabetes, autoimmune disease, previous organ transplant and/ or being unable to use contraception [8].

Study Design: This single center, prospective observational study was conducted at KRL hospital from August 2010 to August 2012. Patients who accepted the treatment were included in the study. The response to treatment was evaluated on the basis of Sustained Virological Response and End of Treatment Response (ETR). The frequency of decline in hematological cell lines was observed prospectively and analyzed for its relation with achieving a good or poor response rate. Individuals ending up as Complete Responders (CR) were the ones with good response rate while Non-Responders (NR) and Relapsers (R) were the ones with poor response.

MATERIALS AND METHODS

A total of 129 non-consecutive, treatment naïve, HCV RNA positive patients with chronic hepatitis C meeting the inclusion and exclusion criteria were given conventional interferon alpha 2b (3 Million IU) thrice weekly plus ribavirin (1000- 1200 mg/day) for 24 weeks. Treatment was stopped in 4 individuals due to serious side effects. 10 individuals either did not come for follow-up or had missing data and were excluded from the study results. A total of 115 individuals were able to complete the total duration of therapy with regular follow-up and thus included.

Baseline variables recorded at first presentation included age, gender, Body Mass Index (BMI), baseline levels for Alanine aminotransferase (ALT), hemoglobin, leukocyte and platelet count. At each visit blood cell counts were measured and recorded. BMI was calculated using the patient's height and weight according to the formula BMI (kg/m^2) = (weight [kg])/ (height [m]) ^{2}.

Dosage of ribavirin was determined by body weight (1000 mg /day in patients less than 75 Kg; 1200 mg/day in patients greater than or equal to 75 Kg) [8]. During treatment patients were assessed as out-patients at 0, 2, 6, 12, 18 and 24 weeks and other causes of cytopenias ruled out by a detailed history, thorough examination and relevant investigations. Response to treatment was assessed via ETR at 24 weeks and via SVR at 48 weeks, both with Qualitative PCR for HCV RNA having lower limit of detection as 50 IU/ml [8]. PCR was carried out by Nested PCR. Patients who received 80% of conventional dose and duration of therapy were declared to have completed treatment [8]. ETR was defined as negative qualitative PCR at end of treatment while SVR was defined as negative PCR six months after completion of therapy. Those achieving ETR and/ or SVR were designated as Complete Responders at respective points in time. Patients with positive PCR at end of treatment and also six months after treatment completion were declared as Non-Responders, whereas those with positive PCR at end of treatment and negative PCR, six months after completion of therapy were defined as Late Responders.

Break-Through Non-Responders (BTNR) were the ones having reappearance of detectable HCV RNA once eradicated while on therapy. Relapse was defined as negative end of treatment PCR but positive PCR after six months of completion of treatment. Definitions used were as per AASLD (American Association for the Study of Liver Diseases) guidelines [9].

Study variables for predicting response to treatment included new onset anemia, thrombocytopenia and/ or leukopenia during the course of interferon therapy. For the purpose of this study, anemia was defined as Hb < 120 g/l for women and < 135 g/ l for men. Thrombocytopenia was defined as a platelet count <150 x 109/ l & leukopenia was defined as a leukocyte count < 4x109/ l. Cut-off values for all cytopenias were in accordance with international standards.

Statistics: Sample size was determined for hypothesis testing for the population proportion using WHO SS calculator [10]. It was calculated to be 72 while keeping level of significance at 5%, power of test at 90% and reported frequency of anemia at 39-56% [4, 11]. A non-consecutive, non-probability sampling technique was used for patient selection.

All analyses were performed using SPSS version 16 (SPSS Inc. USA). Data variables were put in SPSS. Percentage, mean values and standard deviations for baseline variables were calculated using simple descriptive statistics. For univariate analysis, cytopenias were arranged as qualitative variables and assessed to be either present or absent. Their percentages and p values were calculated using descriptive statistics and chi-square test respectively. Data variables were then arranged and assessed using multivariate analysis and results were calculated by forward stepwise logistic regression. P value < 0.05 was taken as statistically significant.

RESULTS

Male individuals constituted 31.3 % (n=36) of the population while 68.7% (n=79) were females. Mean age was 44.18 ± 8.81 years. Mean BMI was 27.55 ± 7.5 kg/ m2. Mean baseline ALT was 72.4 ± 61.15 IU/ l. Mean hemoglobin at baseline was 13.2 ± 1.96 g/ l, leukocyte count was 6.73 ± 1.8 x 109/ l while platelet count was 226.07 ± 81.72 x 109 / l. Out of the total population, 30.4% (n=35) individuals developed anemia, 32.2% (n=37) experienced thrombocytopenia while leukopenia was seen in 50.4% (n=58). ETR was achieved by 80.9% (n=93) while SVR by 58.3% (n=67). (Fig 1) Only leukopenia was a significant predictor of achieving better SVR on univariate analysis (p=0.03) but was insignificant on multivariate analysis (p=0.28). None of the other cell lines was found to be a significant predictor of either SVR or ETR on univariate as well as multivariate analysis (p>0.05) (Table 1).

DISCUSSION

Hepatitis C virus is a major cause of chronic liver disease infecting more than 170 million people worldwide [12]. Conventional Interferon and ribavirin combination therapy are standard of care for chronic hepatitis C in Pakistan [3]. The use of these drugs has been correlated

Table 1: Hematological Parameters as Predictor of Response to Interferon Combination Therapy

Hematological parameters as predictor of ETR					
				P value	
Parameter	CR	BTNR	NR	Univariate	Multivariate
Anemia	91.4 (n=32)	2.9% (n=1)	5.7% (n=2)	0.11	0.49
Thrombocytopenia	81.1% (n=30)	2.7% (n=1)	16.2% (n=6)	0.99	
Leukopenia	82.8% (n=48)	13.8% (n=8)	3.4% (n=2)	0.63	
Hematological parameters as predictor of SVR					
				P value	
Parameter	CR	R	NR	Univariate	Multivariate
Anemia	62.9% (n=22)	28.6% (n=10)	8.6% (n=3)	0.14	0.28
Thrombocytopenia	64.9% (n=24)	18.9% (n=7)	16.2% (n=6)	0.49	
Leukopenia	69% (n=40)	13.8% (n=8)	17.2% (n=10)	0.03*	

Hematological parameters as predictor of response to treatment with interferon combination therapy. ETR=End of Treatment response; SVR=Sustained Virological Response; CR=Complete Responders; BTNR=Break-Through Non-Responders; NR=Non-Responders; R=Relapsers. *=statistically significant.

FIGURE 1A

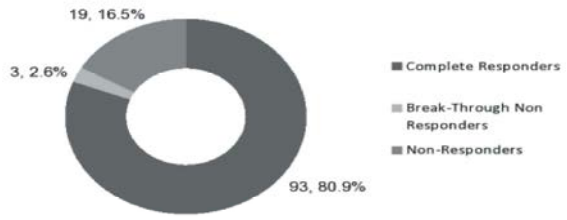

FIGURE 1B

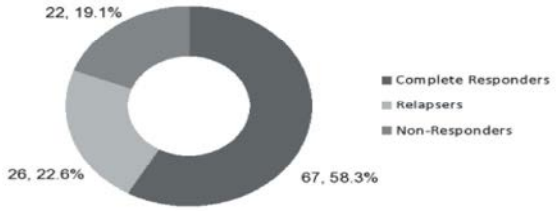

Fig. 1: Response to interferon therapy at ETR and SVR stage shown as total number of individuals and their percentages in respective category. Outcome at ETR stage. Figure 1B: Outcome at SVR stage. ETR=End of Treatment response; SVR=Sustained Virological Response.

with a range of adverse effects, including influenza like symptoms, hematological changes and neuropsychiatric disturbances [13]. The effect on hematological cell lines has been further studied for predicting a better or adverse viral response to this combination therapy [5].

In our study, SVR was achieved by 58.3% (n=67) while ETR by 80.9% (n=93) as a whole. (Fig 1) Hung *et al.* (2006) agreed that anemia positively predicts SVR. SVR and time of severe anemia are linearly correlated with each other [4]. But Turbide *et al.* (2008) reported that drop in hemoglobin or lowest hemoglobin level cannot predict SVR [5]. According to our study 30.4% (n=35) individuals developed anemia. Neither the SVR nor ETR was found to be significantly related to anemia as an independent predictor ($p>0.05$) (Table 1).

The role of leukopenia as a predictor of SVR has been declared controversial by different researchers [5,6]. Martinez-Camacho *et al*. (2011) have reported lymphocytosis during interferon therapy to be a poor predictor of SVR while Turbide *et al*. (2008) concluded that a significant drop in WBC and neutrophil values is a predictor of failure to achieve an SVR [5, 6]. In our study, leukopenia did not affect ETR significantly ($p=0.63$) but the results of this study found it to be a positive predictor of achieving an SVR ($p=0.03$*) on univariate analysis.

We also noted that there is no change in the rate of response with effect of thrombocytopenia ($p=>0.05$) (Table1); quite similar to the researchers who have observed thrombocytopenia to be an insignificant predictor of response [5]. In addition, Taniquchi *et al*. (2006) have shown thrombocytopenia to be a non-invasive marker of hepatic fibrosis [7]. This also suggests poor predictive value of thrombocytopenia for achieving better response rate.

Non-responder and Relapser status was found not to be related with decline in any of the cell lines during treatment ($p=>0.05$). (Table1) This indicates poor predictive value of cytopenias for defining a worse outcome. Several studies have argued that significant treatment-induced cytopenia results in dose reductions to less than 80%, leading to lower SVR rates [5]. Poorer outcomes in such studies may be related to inadequate dosing. Results for our study however are based on the fact that we included the individuals who received at least 80% of the drug dose making the results more reliable.

Combining the effect of decline in all of the three cell lines, multivariate analysis showed that anemia, thrombocytopenia and leukopenia were all insignificant predictors of response rate to interferon combination therapy. (Table 1) This finding is of particular importance because the study on these parameters in South-East Asia has been very limited in the past. Moreover, viral characteristics in this part of the world also differ, i-e-, genotype 3 is more common than other genotypes contrary to rest of the world [14, 15]. We would, therefore, like to suggest that interferon induced cytopenia is a poor predictor of response to therapy in Pakistan. Clinicians should not rely on these parameters for either changing the treatment of their patients or discontinuing the therapy.

CONCLUSIONS

Decline in hematological cell lines during treatment of HCV genotype 3 in Asians using conventional interferon combination therapy is not associated with response rate. Leukopenia however, may have some effect on the outcome as an independent predictor.

Conflict of Interest: The authors declare that they do not have any conflict of interest.

REFERENCES

1. Serfaty, L., H. Aumaître, O. Chazouillères, A.M. Bonnand, O. Rosmorduc, R.E. Poupon and R. Poupon, 1998. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. Hepatology, 27(5): 1435-40.
2. Alter, H.J. and L.B. Seeff, 2000. Recovery, persistence and sequelae in hepatitis C virus infection: a perspective on long-term outcome. Semin Liver Dis., 20(1): 17-35.
3. Cavalletto, L., L. Chemello, C. Donada, P. Casarin, F. Belussi, E. Bernardinello, F. Marino, P. Pontisso, A. Gatta and A. Alberti, 2000. The pattern of response to interferon alpha (alpha-IFN) predicts sustained response to a6-month alpha-IFN and ribavirin retreatment for chronic hepatitis C. TVVH Study Group. J Hepatol., 33(1): 128-34.
4. Hung, C.H., C.M. Lee, S.N. Lu, J.H. Wang, C.H. Chen, T.H. Hu, K.M. Kee, K.C. Chang, P.L. Tseng, Y.H. Yen and C.S. Changchien, 2006. Anemia associated with antiviral therapy in chronic hepatitis C: incidence, risk factors and impact on treatment response. Liver Int ., 26(9): 1079-86.
5. Turbide, C., C. Soulellis, M. Deschênes and N. Hilzenrat, 2008. Does a rapid decline in the hematological and biochemical parameters induced by interferon and ribavirin combination therapy for the hepatitis C virus predict a sustained viral response? Can J. Gastroenterol., 22(2): 149-52.
6. Martinez-Camacho, A., V.I. Khaoustov, E. Adam, D.E. Lewis, S. Tavakoli-Tabasi and B. Yoffe, 2011. Lymphocytosis as a predictor of poor response to treatment of hepatitis C. Clin Res Hepatol Gastroenterol., 35(1): 34-40.
7. Taniguchi, H., Y.I. Wasaki, A. Fujiwara, K. Sakaguchi, A. Moriya, P.C. Yu, T. Aakaki, S. Fujioka, H. Shimomura and Y. Shiratori, 2006. Long-term monitoring of platelet count, as a non-invasive marker of hepatic fibrosis progression and/or regression in patients with chronic hepatitis C after interferon therapy. J. Gastroenterol Hepatol., 21(1 Pt 2):281-7.
8. Sarwar, S., A.A. Khan and S. Tarique, 2011. Pegylated vs. Standard Interferon in Combination with Ribavirin for Genotype 2 and 3 in Chronic Hepatitis C: 10 Years Experience in Local Population. Annals of KEMU, 17(2): 108-112.
9. Swan, T. and J. Curry, 2009. Comment on the updated AASLD practice guidelines for the diagnosis, management and treatment of hepatitis C: treating active drug users. Hepatology, 50(1): 323-4.
10. Lwanga, S.K. and S. Lemeshow, 1991. Sample Size Determination in Health Studies: A practical manual. World Health Organization. Geneva [Internet]. [updated 1991]. Available from: http://www. tbrieder. org/ publications/ books _ english/ lemeshow _ samplesize. pdf.
11. Iqbal, M., A. Waheed and S. Qureshi, 2012. Frequency of anemia and its association with outcome (Sustained Virological Response) in patients of chronic hepatitis C genotype 3, treated with standard interferon alpha 2a and ribavirin. Pak. J. Pharmacol., 29(2): 23-28.
12. Patel, K., A.J. Muir and J.G. McHutchison, 2006. Diagnosis and treatment of chronic hepatitis C infection. BMJ, 332(7548): 1013-7.
13. Sleijfer, S., M. Bannink, A.R. Van Gool, W.H. Kruit and G. Stoter, 2005. Side effects of interferon-alpha therapy. Pharm World Sci. Dec., 27(6): 423-31.
14. Attaullah, S., S. Khan and I. Ali, 2011. Hepatitis C virus genotypes in Pakistan: a systemic review. Virol J., 8: 433.
15. Yu, M.L. and W.L. Chuang, 2009. Treatment of chronic hepatitis C in Asia: when East meets West. J. Gastroenterol. Hepatol ., 24(3): 336-45.