ORIGINAL ARTICLE

RESPONSE OF DIFFERENT HCV GENOTYPES TO INTERFERON THERAPY IN DIFFERENT AGE GROUPS OF CHRONIC HEPATITIS-C PATIENTS

Sajjad Iqbal, Khalil-Ur-Rahman, Munir Ahmad Sheikh, Muhammad Arshad*

Department of Chemistry and Biochemistry, *Department of Microbiology University of Agriculture, Faisalabad, Pakistan

Background: Although new Pegylated Interferon is available, yet the conventional Interferon with the combination of Ribavirin is still the therapy of choice to treat the Hepatitis C patients. This study was conducted to investigate the response of different HCV genotypes in different age groups of chronic Hepatitis C patients treated with conventional Interferonα-2b (IFNα-2b) plus Ribavirin (RBV). **Methods:** In this cross sectional observational study a total of 520 Hepatitis C patients infected with different HCV genotypes meeting the study criteria were included from August 2010 to January 2013. End of treatment response (ETR), sustained virological response (SVR) and the association of patient's age with treatment response were evaluated. ETR and SVR were defined as absence of HCV RNA and normal ALT level at the end of therapy and 12 months after the termination of therapy respectively. **Results:** Out of 520 cases 388 (74.62%) showed ETR. The SVR was observed in 290 (89.23%) out of 325 ETR responders. ETR was higher in males (76.14%) than females (72.77%) while the SVR was almost same in both sex. The highest ETR was noted in genotype 3 (81.15%). The old patients exhibited lower ETR and SVR than youngers in the present study. **Conclusions:** The properly managed conventional Interferon therapy was effective for Hepatitis C patients infected with genotype 2 and 3 with age <40 years as compared to those patients infected with genotype-1 and 4 or had age >40 years.

Keywords: Chronic Hepatitis C, Interferon plus Ribavirin, ETR, SVR.

Ayub Med Coll Abbottabad 2014;26(3):310–5

INTRODUCTION

Currently two FDA approved standard treatment combinations are available. First is the recombinant Interferon alpha (IFNα) with Ribavirin which is also known as conventional Interferon (C-INF) therapy and the other is Pegylated Interferon alpha (PegIFNα) with Ribavirin.[1–6] Although current studies and clinical trials have proven that the response of Hepatitis C patients to pegIFNα-2a or pegIFNα-2b is much better than conventional IFN (C-INF)[7], however the C-INF is still the drug of choice to treat the Hepatitis-C patients in the poor countries including Pakistan.[8,9] It may be because of financial reasons as its cost is lower than pegIFNα which is expensive and beyond the reach of poor patients. Secondly the frequent existence of HCV genotype 3 in Pakistan and other Asian countries that have a good response to conventional Interferon therapy.[9–11] The Society of gastroenterology and GI endoscopy has also recommended the use of C-IFN in Pakistan to treat the HCV patients infected with genotype-3.[9,12]

Some patients who showed response at the completion of therapy (negative HCV RNA and normal ALT level) relapsed within the six months of the therapy termination (Positive HCV RNA and elevated ALT level).[5,13] Many viral, immunological or host associated factors affect the efficacy of HCV infected patient's response to Interferon therapy.[14] In Pakistan the patients infected with HCV genotype-2 and 3 exhibit 70–80% end of therapy response (ETR) to combination therapy (IFNα plus Ribavirin). On the other hand the patients infected with genotype 1and 4 show relatively low ETR (20–45%) to IFNα plus Ribavirin combination therapy.[12,13] Similarly, the ETR of HCV genotype 2 and 3 infected patients from other parts of the world has been reported up to 72%, while the patients infected with genotype 1 and 4 show low ETR (13%).[15,16]

The available literature on long term biochemical and virological outcome as well as benefits of Interferon plus Ribavirin combination therapy in Pakistan was scanty and the present study was planned to probe into these arenas.

MATERIAL AND METHODS

This cross sectional observational study was conducted from August 2010 to January 2013 at Shalamar Hospital, Lahore, Pakistan. A total of 520 patients willing to take part in the study and provided all the required information were included in the present study. 325 participants who showed the end of therapy response (ETR) were followed up for one year after the termination of therapy to see the virological sustained response. Pre-treatment HBV vaccination was started to all the HCV infected patients who were negative for HBsAg (by ELISA) to reduce the chances of HBV co-infection. Treatment was given under qualified gastroenterologists and hematologists.

Patients with age range 10–60 years (Mean=35±6) and never received interferon therapy before and were HCV RNA positive by polymerase-

chain-reaction (PCR) were included in this study. Pre-treatment data (Address, contact number, family status, age, sex, previous investigations) were taken. All the patients were anti-HCV positive by ELISA and the current infection was confirmed by HCV RNA detection. Pre-treatment ALT level, HCV genotyping, and HCV RNA quantification was done.

The patients co-infected with HBV or HIV were excluded from the study to see the therapy response only in HCV positive patients. The patients with thrombocytopenia (less than 50,000 platelets per cubic millimeter), anemia (lower than 10g of hemoglobin per deciliter in women and lower than 11g per deciliter in men) and those who had active TB disease were excluded from the study. All the patients who had decompensate liver disease and serum creatinine level more than 1.5 times the upper limit of normal level were also excluded. The patients with poorly controlled psychiatric or diabetes disease were also not taken in the present study.

Patients were divided into two groups. In first group those patients infected with HCV genotypes other than 1 (1a, 1b) and 4 were included and in the second group, patients infected with genotype 1 (1a, 1b) and 4 were taken. First group patients received 3MU recombinant IFNα-2b thrice a week plus Ribavirin (1000 mg/day in patients under or equal to 75kg body weight or 1200 mg/day above 75 kg body weight into two divided doses daily) for a total of 24 weeks. In second group treatment was same but extended to 48 weeks as per treatment protocols.

For the detection of HCV RNA and to measure the viral load Real-time PCR technique was used. Amplification was done with commercially available HCV RNA Real-time amplification kit (Robogene® HCV RNA qualitative and quantification kit: AJ ROBOSCREEN Germany). The detection limit of the kit was <100 IU/ml or <200 copies/ml. The HCV RNA was isolated by column based, Roboscreen RNA isolation kit (Instant virus RNA kit: AJ Roboscreen Germany).

For HCV genotyping, HCV RNA was extracted from 250µL serum with the TRI Reagent RNA isolation kit (TRI REAGENT-LS, TS 120, by the Molecular Research Center, USA) according to the kit protocol.

First the complementary DNA was synthesized using RT-PCR for HCV genotyping. For that 10µL isolated RNA with two hundred units of Moloney-murine leukemia virus reverse transcriptase enzyme (M-MLV RT) and 1M outer anti-sense primer reported before[17] was used to convert RNA to cDNA according to the manufacturer's instructions (Fermentas®). Then the cDNA was amplified using the type-specific primers[17]. The assay was established in the core region of the HCV genome that includes two amplification rounds after the preparation of cDNA. In the first round with a pair of outer sense and anti-sense primers a large fragment was amplified. In the second round inner portion of the first round fragment was amplified using type specific primers. The primers were designed in this way that amplified product for a specific genotype had specific fragment size. The second round amplified products were run on 2.5% agarose gel stained with ethedium bromide. The gel was analyzed on UV light. HCV genotypes were differentiated on the bases of amplified fragment size comparing with 100 bps DNA ladder marker.

RESULTS

A total of 520 patients (235 females and 285 males) chronically infected with different HCV genotypes were enrolled to treat and see the response. The age range was 10–60 years (Mean=35±6) both in females and males (Table-3 & 4). Out of 520 HCV patients 26 were infected with HCV genotypes 1 (1a+1b) & 4 and 429 with genotypes 2 (2a+2b) & 3 (3a+3b). Fifty cases were untypeable and 15 patients were infected with mixed (more than one) HCV genotypes as demonstrated in table-1.

Out of 520 cases 74.62% (n=388) showed the end of therapy response (ETR) and 25.38% (n=132) failed to eradicate the virus from their bodies (Table-1). To see the sustained virological response (SVR) in the end of therapy responders a total of 325 patients were followed up for 12 months after the completion of therapy. The SVR was examined in 89.23% (n=290) while 10.77% (n=35) relapsed (table-2). ETR and SVR were associated with absence of HCV RNA and normal ALT level at the end of therapy and during the follow up period respectively.

The ETR was higher in males (76.14%) than females (72.77%) (Table-3). The sustained virological response (SVR) was almost same both in males and females, i.e., 88.71% in males and 89.93% in females (Table-4). In major genotypes, the highest ETR was noted in genotype 3 (81.15%) and genotype 2 (71.43%). The ET response of genotype 1 and 4 was 44.44% and 37.50% respectively. Untypeable and mixed genotypes demonstrated 56% and 46.67% end of therapy response respectively. When the ETR was explored in HCV subtypes, the highest percentage was present in subtype 3b (85.53%) and the lowest in subtype 1a (38.46%), while in subtype 1b, 2a, 2b and 3a it was noted 60%, 70%, 76.92% and 78.04% respectively (Table-1).

As illustrated in fig-1 the ETR was observed 25% in those mixed subtypes in which one or both belongs to genotype-1 or 4. The ETR was 85.71% in those mixed subtypes in which both

belong to genotype 2 or 3. The same situation was observed in SVR response that was 50% in those mixed subtypes in which one or both belongs to genotype 1 or 4, while 83.33% in those subtypes in which both belongs to genotypes 2 or 3 (Figure-2). Gradually decreasing trend of ETR and SVR was observed with the increase of age range both in females and males HCV infected patients. The aged patients exhibited lower ETR and SVR than younger in the present study (Table-3 & 4).

The collective ETR of genotype 1 and 4 (1+4) was 42.30%, while in genotype 2 and 3 (2+3) it was 79.72%.The ETR difference between these two groups was significant (p<0.005). The same situation was observed in their SVR, i.e., genotypes 1+4 exhibited 33.33% collective SVR and in genotype 2+3 SVR was 93.08%.

Table-1: ETR of different HCV genotypes and subtypes

Genotypes	Subtypes	Total	Responding	Non-Responding
1	1a	13	5 (38.46%)	8 (61.54%)
	1b	5	3 (60%)	2 (40%)
2	2a	50	35 (70%)	15 (30%)
	2b	13	10 (76.92%)	3 (23.08%)
3	3a	214	167 (78.04%)	47 (21.96%)
	3b	152	130 (85.53%)	22 (14.47%)
4	4	8	3 (37.50%)	5 (62.50%)
Untypeable	Untypeable	50	28 (56%)	22 (44%)
Mixed	Mixed	15	7 (46.67%)	8 (53.33%)
Total		520	388 (74.62%)	132 (25.38%)

Table-2: SVR of different HCV genotypes and subtypes

Genotypes	Subtypes	Total	Sustained response	Relapsed cases
1	**1a**	5	2 (40%)	3 (60%)
	1b	3	1 (33.33%)	2 (66.67%)
2	**2a**	25	22 (88%)	3 (12%)
	2b	7	5 (71.43%)	2 (28.57%)
3	**3a**	170	162 (95.29%)	8 (4.71%)
	3b	87	80 (91.95%)	7 (8.05%)
4	**4**	1	00 (0.0%)	1 (100%)
Untypeable	**Untypeable**	22	15 (68.18%)	7 (31.82%)
Mixed	**Mixed**	5	3 (60%)	2 (40%)
Total		325	290 (89.23%)	35 (10.77%)

Table-3: Age and gender wise distribution of ETR

Age in Years	Total cases			ETR		
	Females	Males	Total	Females	Males	Total
10–20	18	14	32	15 (83.33%)	12 (85.71%)	27 (84.38%)
21–30	66	54	120	53 (80.30%)	4583.33%	98 (81.67%)
31–40	71	107	178	57 (80.28%)	87 (81.31%)	144 (80.90%)
41–50	59	93	152	36 (61.02%)	63 (67.74%)	99 (65.13%)
51–60	21	17	38	10 (47.62%)	10 (58.82%)	20 (52.63%)
Total	235	285	520	171 (72.77%)	217 (76.14%)	388 (74.62%)

Table-4 Age and gender wise distribution of SVR in end of therapy responders

Age Yrs	EOT Response			SVR		
	Females	Males	Total	Females	Males	Total
10–20	11	9	20	10 (90.91%)	9 (100%)	19 (95%)
21–30	46	38	84	43 (93.48%)	36 (94.74%)	79 (94.05%)
31–40	45	75	120	42 (93.33%)	67 (89.33%)	109 (90.83%)
41–50	31	58	89	26 (83.87%)	50 (86.21%)	76 (85.39%)
51–60	6	6	12	4 (66.67%)	3 (50%)	7 (58.33%)
Total	139	186	325	125 (89.93%)	165 (88.71%)	290 (89.23%)

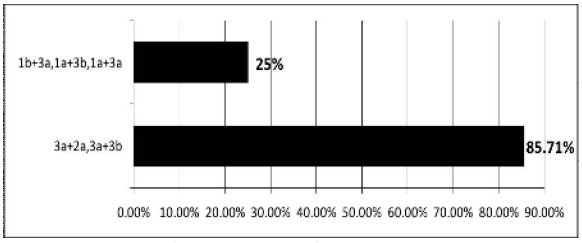

Figure-1: ETR of two groups of HCV mixed subtypes

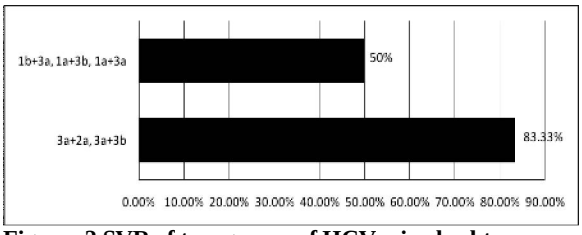

Figure-:2 SVR of two groups of HCV mixed subtypes

DISCUSSION

In the present study conventional Interferon combination therapy (Interferon-α 2b plus Ribavirin) was used. For that 520 Hepatitis C patients infected with different HCV genotypes were enrolled. Out of those 388 (74.62%) showed the end of therapy response (ETR) and 132 (25.38%) failed to eradicate the virus from their body (Table-1). ETR was associated with normal ALT and undetectable HCV RNA at the end of therapy. The SVR was associated with sustained normal ALT and undetectable HCV RNA 12 months after the end of therapy that was also described previously.[18,19] The ETR was high in the present study as compared to previous studies done by Idrees and Riazudin in 2009 where the ETR was observed in 67% and was in accordance with other local and international studies.[20–24] Compared to other studies reported by Nadeem *et al*[25] and Mahsud *et al.*[26] where the ETR was 84% and 81.12% respectively the ETR rate in this study was low.

According to the findings of present study, SVR was achieved by 89.23% (290/325), while

10.77% (35/325) failed to sustain their response and relapsed during the follow up period (post therapy) in which the late increase in serum ALT levels was seen and the HCV RNA also became positive. The relapse rate of this study was higher than Khokhar,[27] i.e, 4.9% and lower than Jadoon *et al*[28] i.e., 15.6%; Ahmed *et al*, i.e., 37% and Bhutta *et al*[29] i.e., 39.23%.

Sustained virological response (SVR) of the Hepatitis C patients enroled in the present study was low 290 (89.23%) as compared to previous study where it was 96% done by Patrick *et al*[30] and higher than Kalantari *et al*[31]; Muhammad *et al*[32] and Ahmed *et al*[20] where the SVR was 75.14%, 78.85% and 63% respectively. Out of 325 ET responders, 290 (89.23%) were able to sustained their response 12 months after the therapy termination and two relapsed (one after 9 months and the other after 11 months of the therapy completion). The percentage of late relapsed cases (after 6 months of the end of therapy) was very low, i.e., 0.68% (2/292) it indicates that the patients who remained HCV RNA negative up to 6 months of post therapy period had more chance to eradicated the virus from their body permanently and could be considered sustained responders.

Sustained virological response (SVR) reported from European countries was lower than this region. SVR reported by Ming *et al*[33] and John *et al*[34] was 34% and 31% respectively. Whereas SVR reported from Pakistan was significantly high, i.e., 96% reported by Patrick *et al*[30]; 75.14%, 78.85% and 63% reported by Kalantari *et al*[31]; Muhammad *et al*[32] and Ahmed *et al*[20] respectively while in the present study the SVR was 89.23%. This discrepancy of low and high response rate may be because of the epidemiological difference of the HCV genotypes. In Pakistan the most common HCV genotypes are 2 and 3[35–37] which are good responders to Interferon combination therapy as compared to genotype 1 and 4 which are mostly found in European countries.[38–40] These findings also support the fact that HCV genotyping assessment in therapy management should be mandatory.

In different age groups the rate of response to therapy was different. Younger patients who were less than or equal to 40 years of age showed better ETR and SVR (81.52% and 92.41%) than the patients who were more than 40 years of age (ETR=62.63% and SVR=82.17%) (Table-3 and 4). ETR and SVR were investigated 71.2% and 57.6% respectively by Idrees and Riazudin (2009)[12] in the same age group which was lower than this study. Giorgio *et al*,[41] also found better response in the same age group. With the increasing trend of age the ETR response to Interferon plus Ribavirin combination therapy decreased (Table-3), which has also been noted in other local studies.[20,42]

The ETR was higher in males i.e., 76.14% than females, i.e., 72.77%, while the SVR was almost same in both the sexes (88.71% in males and 89.93% in females) (Table-3 and 4), which suggest that males had a better response than females. The results of present study regarding gender wise ETR response were contrasted to previous studies[12,20,43,44] where they found significantly better response in females than males.

The patients infected with subtypes of HCV genotype-1 (1a, 1b) exhibited the lower ETR than those infected with subtypes that belongs to genotype-2 and 3 (2a, 2b, 3a and 3b) (Table-1). The end of therapy response (ETR) in subtypes 1a, 1b and genotype-4 was 38.46%, 60% and 37.50%, while the ETR in subtypes 2a, 2b, 3a and 3b was noted 70%, 76.92%, 78.04% and 85.53% respectively. These results were in accordance with the view that HCV subtypes 1a, 1b and 4 are associated with a poor rate of response to Interferon-alpha therapy as compared to subtypes 2a, 2b and 3a, 3b.[13,20,45] So the findings of the present study show that the response rate for Interferon therapy was highly associated with the genotypes and it revealed that in HCV therapy management genotypes plays a vital role.

The SVR was also high in subtypes 3a, 3b, 2a and 2b (95.29%, 91.95%, 88% and 71.43% respectively) than 1a, 1b and 4 (40%, 33.33% and 0%) (Table-2). That was a good news for this region where most common subtypes are 2a, 2b and 3a, 3b.[13,44,46,47] It is also important to note that the number of cases of genotype 1a, 1b, 2b and 4 were low which needs more study to evaluate exact results.

Twenty patients infected with untypeable genotypes, were treated on a trial basis for one year and they showed the ETR and SVR 56% and 68.18% respectively. In mixed genotypes the ETR was observed low in which one or both mixed genotypes were 1 or 4 as compared to those in which the both genotypes were non 1 or 4. That indicates that the mixed genotypes which include genotype 1 or 4 in combination should be treated for twelve months according to the standard protocol of therapy for these genotypes (1 or 4), and the mixed genotypes in which both types are non 1 or 4 may be treated for six months.

CONCLUSION

In conclusion, treatment response was highly associated with the HCV genotype and the age of the patient. The response of HCV genotypes 2 and 3 was better to conventional Interferon therapy in younger patients as compared to genotype 1 and 4 and older patients. It revealed that in HCV therapy management viral genotype and the patient's age play a vital role. So to treat the Hepatitis C patients infected with

genotype 1 and 4 other treatment options like Pegylated Interferon therapy should also be taken under consideration to get better results.

REFERENCES

1. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, *et al*. Interferon signaling and treatment outcome in chronic Hepatitis C. Proc Natl Acad Sci U S A 2008;105:7034–9.
2. Manns MP, McHutchison LG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, *et al*. PegInterferon alfa-2b plus Ribavirin compared with Interferon alfa-2b plus Ribavirin for initial treatment of chronic Hepatitis C: a randomised trial. Lancet 2001;358:958–65.
3. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, *et al*. PegInterferon alfa-2a plus Ribavirin for chronic Hepatitis C virus infection. N Engl J Med 2002;347:975–82.
4. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, *et al*. PegInterferon-alpha2a and Ribavirin combination therapy in chronic Hepatitis C: a randomized study of treatment duration and Ribavirin dose. Ann Intern Med 2004;140:346–55.
5. Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC Jr, Perrillo RP, *et al*. Treatment of chronic Hepatitis C with recombinant Interferon alpha. A multi-center randomized, controlled trial. Hepatitis interventional therapy group. N Engl J Med 1989;321:1501–6.
6. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with pegInterferon alfa-2b plus Ribavirin in patients with chronic Hepatitis C. Hepatology 2003;38:645–52.
7. Vigani AG, Goncales ES, Pavan MH, Genari F, Tozzo R, Lazarini MS, *et al*. Therapeutic effectiveness of biosimilar standard interferon versus pegylated interferon for chronic hepatitis C genotypes 2 or 3. Braz J Infect Dis 2012;16:232–6.
8. Hadziyannis SJ, Cheinquer H, Morgan T. Peg Interferon alpha-2a (40 KD) in combination with Ribavirin efficacy and safety results from phase 3, randomized double blind, multicenter study examining effect of duration and Ribavirin dose. J Hepatol 2002;36(1):3–14.
9. Karayiannis P. The Hepatitis C virus NS3/4A protease complex interferes with pathways of the innate immune response. J Hepatol 2005;43:743–5.
10. Zuberi BF, Zuberi FF, Memon SA, Qureshi MH, Ali SZ, Afsar S. Sustained virological response based on rapid virological response in genotype-3 chronic Hepatitis C treated with standard Interferon in the Pakistani population. World J. Gastroenterol 2008;14:2218–21.
11. Peribanez-Gonzalez M, da Silva MH, Vilar FC, Seixas-Santos Nastri AC, Ferriera PA, Focaccia R, Correa MCM. Response predictors and clinical benefits of hepatitis C retreatment with pegylated interferon and ribavirin in HIV/HCV coinfection. Ann Hepatol 2013;12:228–35.
12. Idrees M, Riazuddin S. A study of best positive predictors for sustained virologic response to Interferon alpha plus Ribavirin therapy in naive chronic Hepatitis C patients. BMC Gastroenterol 2009;9:5.
13. Iqbal S, Rehman K, Dogar ZH, Bashir S, Akhtar MS. Sustained biochemical and virological response of different HCV genotypes to Interferon-alpha plus Ribavirin combination therapy. Pharmacologyonline 2010;2:161–9.
14. de Careaga BO. Predictive factors for response to treatment of chronic Hepatitis C. Ann Hepatol 2006;5:S24–8.
15. Hofmann WP, Zeuzem S, Sarrazin C. Hepatitis C virus resistance mechanisms to Interferon- α based antiviral therapy. J Clin Virol 2005;32:86–91.
16. Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, *et al*. PegInterferon alfa-2a (40 kilodaltons) and Ribavirin in patients with chronic Hepatitis C and normal aminotransferase levels. Gastroenterology 2004;127:1724–32.
17. Ohno T, Mizokami M, Saleh MM, Ohba K, Orito E, Mukaide M, *et al*. New Hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Micro 1997;35:201–7.
18. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, *et al*. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic Hepatitis C. Gastroenterol 2002;123:1061–9.
19. Marcellin P, Boyer N, Gervais A, Martinot M, Pouteau M, Costelnau C, *et al*. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic Hepatitis C and sustained response to Interferon-alpha treatment. Ann Intern Med 1997;127:875–81.
20. Ahmed W, Arif A, Qureshi H, Alam SE, Ather R, Fariha S, *et al*. Factors influencing the response of Interferon therapy in chronic Hepatitis C patients. J Coll Physicians Surg Pak 2011;21:69–73.
21. Shaikh WM, Shaikh MA, Solangi GA, Zuberi BF. Role of Interferon and Interferon plus Ribavirin in the management of chronic Hepatitis C. J Coll Physicians Surg Pak 2002;12:609–12.
22. Farooqi JI, Farooqi RJ. Efficacy of conventional Interferon alpha-2b plus Ribavirin combination in the treatment of chronic Hepatitis C naive patients. Rawal Med J 2005;30:9–11.
23. Manns MP, Wedemeyer H, Cornberg M. Treating viral Hepatitis C: efficacy, side effects and complications. Gut 2006;55:1350–9.
24. Herrine SK, Rossi S Navarro VJ. Management of patients with chronic Hepatitis C infection. Clin Exp Med 2006;6:20–6.
25. Nadeem A, Hussain MM, Aslam M, Hussain T, Butt IF, Ali Khan S, *et al*. Association of response to combined Interferon alpha-2b and Ribavirin therapy in patients of chronic Hepatitis C with serum alanine aminotransferase levels and severity of the disease on liver biopsy. J Ayub Med Coll Abbottabad 2009;21(2):103–6.
26. Mahsud I, Khan RD, Khan M, Hameed K. Response of Hepatitis C patients to alpha Interferon and Ribavirin combination therapy. Gomal J Med Sci 2008;6(2):65–8.
27. Khokhar N. Late relapse in chronic hepatitis C after sustained viral response to interferon and Ribavirin. J Gastroenterol Hepatol 2004;19:471–2.
28. Jadoon SM, Jadoon S, Muhammad I. Response to standard Interferon a2b and Ribavirin combination therapy in chronic Hepatitis C treatment naive patients. J Ayub Med Coll Abbottabad 2010;22(4):164–6.
29. Bhutta S, Wasimuddin, Muzamil J. Short duration therapy with standard Interferon and Ribavirin in chronic Hepatitis C genotype 3a patients. Is it too short? Ann Pak Inst Med Sci 2011;7(2):86–9.
30. Marcellin P, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, *et al*. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic Hepatitis C and sustained response to Interferon-alpha therapy. Ann Int Med 1997;127:875–81.
31. Kalantari H, Kazemi F, Minakari M. Efficacy of triple therapy with Interferon alpha-2b, Ribavirin and Amantadine in the treatment of naive patients with chronic Hepatitis C. J Res Med Sci 2007;12:178–85.
32. Muhammad N, Jan MA, Rahman N. Outcome of combine interferon-ribavirin in the treatment of chronic Hepatitis C. J Coll Physicians Surg Pak 2004; 14:651-653.
33. Lai MY, Kao JH, Yang PM, Wang JT, Chen PJ, Chan KW, *et al*. Long term efficacy of Ribavirin plus Interferon alpha in the treatment of chornic Hepatitis C. Gastrointerology 1996;111:1307–12.
34. John M, Flexman J, French MA. Hepatitis C virus-associated Hepatitis following treatment of HIV-infected patients with

HIV protease inhibitors: an immune restoration disease. AIDS 1998;12:2289–93.
35. Idrees M, Riazuddin S. Frequency distribution of Hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infect Dis 2008;8:69.
36. Ahmad W, Ijaz B, Javed FT, Jahan S, Shahid I, Khan FM, *et al*. HCV genotype distribution and possible transmission risks in Lahore, Pakistan. World J Gastroenterol 2010;16:4321–8.
37. Iqbal S, Ahmad R, Yousaf MH, Mumtaz A, Amine D, Rasool G, *et al*. Assessment of major genotypes and subtypes of Hepatitis C virus. Professional Med J 2007;14:266–71.
38. Burguete-Garcia AI, Conde-Gonzlez CJ, Jimenez-Mendez R, Juarez-Diaz Y, Meda-Monzon E, Torres-Poveda K, *et al*. Hepatitis C sero-prevalence and correlation between viral load and viral genotype among primary care clients in Mexico. Salud Publica Mex 2011;53(Suppl 1):S7–12.
39. Eriksen MB, Jorgensen LB, Krarup H, Laursen AL, Christensen PB, Moller A, *et al*. Molecular and epidemiological profiles of Hepatitis C virus genotype 4 in Denmark. J Med Virol 2010;82:1869–77.
40. Haushofer, AC, Kopty C, Hauer R, Brunner H, Halbmayer WM. HCV genotypes and age distribution in patients of Vienna and surrounding areas. J Clin Virol 2001;20(1-2):41–7.
41. Antonucci G, Angeletti C, Vairo F, Longo MA, Girardi E. Age and prediction of sustained virological response to Hepatitis C virus (HCV) infection treatment based on 28-day decrease in HCV RNA levels. J Infect Dis 2009;200:1484–5.
42. Shiffman ML, Mihas AA, Millwala F, Sterling RK, Luketic VA, Stravitz RT, *et al*. Treatment of chronic Hepatitis C virus in African Americans with genotypes 2 and 3. Am J Gastroenterol 2007;102:761–6.
43. Bakr I, Rekacewicz C, El Hosseiny M, Ismail S, El Daly M, El-Kafrawy S, *et al*. Higher clearance of Hepatitis C virus infection in females compared with males. Gut 2006;55:1183–7
44. Shindo M, Arai K, Sokawa Y, Okuno T. Hepatic Hepatitis C virus RNA as a predictor of a long-term response to Interferon-alpha therapy. Ann Intern Med 1995;122:586–91.
45. Kjaergard LL, Krogsgaard K, Gluud C. Interferon alfa with or without Ribavirin for chronic Hepatitis C: systematic review of randomised trials. BMJ 2001;323:1151–5.
46. Attaullah S, Khan S, Ali I. Hepatitis C virus genotypes in Pakistan: a systemic review. Virol J 2011;8:433–40.
47. Chung RT, Monto A, Dienstag JL, Kaplan LM. Mutations in the NS5A region do not predict interferon-responsiveness in american patients infected with genotype 1b hepatitis C virus. J Med Virol 1999;58:353–8.

Address for Correspondence:
Dr. Sajjad Iqbal Sial, Department of Pathology, Shalamar Hospital, Shalimar link Road Lahore, Pakistan.
Cell: +92-333-6446006
Email: sialbrothers@yahoo.com, sajjadsial123@gmail.com