Increasing resistance to combination therapy among the chronic HCV 3a infected patients in KPK

Ruqiya Pervaiz [1], Ijaz Ali[1], Sajid Ali[2] Najib ur Rehman [1], Farzana[1], Riaz Muhammad [2] , Ahmad ur Rehman Saljoqi[3] Musharaf Ahmad [4]

[1]Institute of Biotechnology and Genetic Engineering, Agricultural University, Peshawar, Khyber Pakhtunkhwa, Pakistan.
[2]Centre for Biotechnology and Microbiology, University of Peshawar, Peshawar, Khyber Pakhtunkhwa, Pakistan.
[3]Department of Plant Protection, , Agricultural University, Peshawar, Khyber Pakhtunkhwa, Pakistan.
[4]Department of Plant Pathology, , Agricultural University, Peshawar, Khyber Pakhtunkhwa, Pakistan.
Vet_sajid@yahoo.com

Abstract: Hepatitis C is highly prevalent in Khyber Pakhtunkhwa (KPK) Pakistan. Combination therapies of Interferon or Pegylated Interferon in combination with ribavirin are currently being used as antiviral options. The main aim of this study was to evaluate the response rate of combination therapy among patients infected with HCV 3a in Khyber Pakhtunkhwa Pakistan. A total of 50 patients (29 male and 21 female) belonging to different regions of KPK were enrolled for the study. All the patients were chronically infected with HCV 3a with elevated LFTs. Out of the total 50 patients, 30 (60%) showed ETR and 20 (40%) were Non Responders (NR). Follow up study indicated that all individuals with ETR developed a sustained virological response (SVR). Early virological response was observed only in 2 patients infected with HCV 3a. The response rate (60%) of combination therapy against HCV 3a infected patients of KPK and FATA is comparatively lower than earlier estimates. Our study supports the clinical observation of increasing resistance to combination therapy being experienced in the case of HCV 3a infections.
[Ruqiya Pervaiz, Ijaz Ali, Sajid Ali, Najib ur Rehman, Farzana, Riaz Muhammad, Ahmad ur Rehman Saljoqi, Musharaf Ahmad. **Increasing resistance to combination therapy among the chronic HCV 3a infected patients in KPK.** *Life Sci J* 2013;10(12s):423-426] (ISSN:1097-8135). http://www.lifesciencesite.com. 72

Keywords: HCV 3a, Sustained Virological Response, Combination therapy, Khyber Pakhtunkhwa

Introduction:

Hepatitis C is a transmissible disease affecting the liver, caused by one of the important Flaviviridae virus HCV that causes acute and chronic hepatitis and can lead to liver cancer. Hepatitis C virus is a positive sense single stranded RNA virus, identified in 1989 [1]. In developing countries Hepatitis C is a major health problem including Pakistan [2]. An estimated 200 million people are infected with HCV worldwide and about 17 million people in Pakistan [3, 4]. HCV has six major genotypes and hundreds of subtypes globally identified so far [5]. Different HCV genotypes have different response rate to alpha interferon therapy [6]. Multiple studies confirm that type 3 is the predominant HCV genotype in Pakistan with a prevalence of between 75–90% and are more responsive to interferon based therapy as compared to the patients having genotype 1 HCV infection [7]. Interferon (IFN) is believed to be the only effective drug used by patients with chronic hepatitis C, but its response rate is less than 20% after 24-48 weeks of monotherapy [5]. Even if combined with ribavirin, the response rate has been reported to be only 40% in the case of some HCV genotypes [4]. HCV is highly prevalent among the population of Khyber Pakhtunkhwa province [8]. Various studies conducted in KPK have reported HCV 3a to be the most abundant type of the virus found among the general population [9, 8]. Various local studies have documented that HCV 3a was highly responsive to combination therapy [10, 11]. However, recently, in clinical practice, Physicians have been observing increasing resistance to combination therapy [IFN & Ribavirin] against HCV 3a infection in Pakistan. This study aimed at finding the response rate of combination therapy among patients chronically infected with HCV 3a in the Khyber Pakhunkhwa province of Pakistan.

Methods:

This study was approved by the ethics committee of the Institute of Biotechnology and Genetic Engineering, Peshawar. Chronically infected hepatitis C Patients of various age groups and locations volunteered for the study. All the registered patients had previously been tested for anti HCV by Immunochromatography and ELISA. Active HCV infection was determined in the case of each patient using RT-PCR as described earlier [8]. Genotype determination was carried out by Type-specific PCR as described elsewhere [6]. Among the confirmed HCV 3a genotype patients, 50 patients (29 male, 21 female, ages 30-65 years) were included in this study. None of the patients were cirrhotic or co-infected with any pathogen other than HCV. The duration of

infection varied in different patients as assessed from the patient's history. All the patients had elevated serum ALT levels. All studied patients received a combination of IFN-α 2a (180µg /week) and Ribavirin (400mg/day) for a total of 24 weeks. The patients were monitored for the presence of active HCV infection and ALT level after 12 and 24 weeks of treatment. Efficacy of treatment was evaluated with normalization of ALT level and absence of active HCV RNA measured at week 12 and 24 which constituted the Early Virological Response (EVR) and the End of Treatment Response (ETR) respectively. Patients who had exhibited an ETR were followed up for one year. PCR was conducted and ALT levels monitored during the follow up period in the case of all subjects.

Results:

A total of fifty patients (29 male and 21 female) having ages among 30 to 65 years were included in this study. All the patients completed the 6 months long combination therapy and had been regularly monitored for LFTs and HCV RNA. At the end of treatment, out of 50 patients, 30 (60%) showed ETR (20 males and 10 females). The percentage of undetectable serum RNA was 2% at week 12 and 60% at week 24. The percentage of ETR achieved was comparatively higher among males as compared to females. The percentage of Sustained Virological Response (SVR) was also observed to be the same as percentage of ETR [Table 1]. The percentage of Non Responders (NR) was dramatically high among females (52.38%) as compared to male (31.03%).

All the enrolled subjects were divided into four age groups. Most favorable response to combination therapy was observed in the case of the youngest age group (25-35 years) followed by 36-45 years and 46-55 years. Non-responders were observed in all age groups except the youngest one (25-35 years). The highest percentage of non-responders was found among the older age group (56-65 years) [Table 2].

Table 1: Patients having negative HCV RNA at week 12, 24 and 48.

Treatment duration /Response Observed	Patients with -ve HCV RNA	Male	Female
12 weeks [EVR]	1 (2%)	1 (2%)	0
24 weeks [ETR]	30 (60%)	20 (68.96%)	10 (47.62%)
48 weeks [SVR]	30 (60%)	20 (68.96%)	10 (47.62%)

Table 2: Response rate of combination therapy in various age groups.

Age Groups	Total patient	Male ETR	Female ETR	NR Male	NR Female
25-35	**12**	**9 (100%)**	**3 (100%)**	_____	_____
36-45	**18**	**6 (60%)**	**5 (62.5%)**	**4 (40%)**	**3 (37.5%)**
46-55	**13**	**3 (42.86%)**	**3 (50%)**	**4 (57.14%)**	**3 (50%)**
56-65	**7**	**0 (0%)**	**1 (16.67%)**	**1 (100%)**	**5 (83.33%)**

Discussion:

Hepatitis C is a serious global health problem. About 170 million people are chronically infected and about three to four million people are newly infected every year. Pakistan has huge burden of Hepatitis C. Pakistan is among the worst affected nations and carries one of the highest burdens of HCV [12]. HCV has six major genotypes and hundreds of subtypes globally identified so far [13]. Different HCV genotypes have different response rate to alpha interferon therapy [14].

Pakistan is among the worst affected nations and carries one of the highest burdens of HCV. Literacy rate is very low and there is lack of an effective disease awareness system due to which the general public is least educated about the pathogenecity, routes of transmission and the proper procedures of diagnosis and treatment. A considerable portion of the population lives on less than a dollar a day. HCV infection, therefore, has become an economic burden on the impoverished people of Pakistan and especially in KPK.

HCV 3a is the predominant genotype prevalent in Pakistan []. In this study we determined the response rate of combination therapy of interferon plus ribavirin against HCV 3a genotype infection. Our results indicated that the average calculated ETR was 60% while 40% subjects with HCV 3a infection did not show a favorable response to combination therapy. Follow up study also indicated that all individuals with ETR developed a sustained virological response (SVR).

It has been already documented that HCV genotypes are linked with the treatment efficacy and duration of IFN-therapy [20]. Various studies have shown that combination therapy showed anti-viral response rate of 80% in genotypes 2-6 and 50% in genotype 1 [15]. Studies conducted in Pakistan have also documented higher ETR and SVR rates (>70%) in patients infected with HCV 3a [16, 17, 18]. ETR

rates among HCV 3a infected patients observed in KPK province is comparatively lower than the previous studies carried out in other provinces of the country. Increasing resistance to combination therapy in the case of HCV 3a infected individuals calls for more investigations with respect to virological aspects of the infection. Mutations that accumulate over times in the HCV genomes may significantly alter the response rates among the affected population. One of the other reasons for the poor response rate among the HCV 3a infected subjects may be the shipment and storage conditions of the recombinant IFN. Sever power breakdown which is being experienced over the past couple of years has further deteriorated the local capacity of the health care units to store peptide drugs at lower temperatures.

In contrast to various local and international studies, male subjects exhibited better response rates (69%) than the female (47%). However, the low response rate may be due to the advanced age of the female subjects as age is also an important factor with respect to the management of chronic hepatitis C. Higher SVR rates have been reported among the Pakistani male population recently [19]. Variable response rates have been documented by various investigators in the case of various age groups [20]. In our study, we observed a trend of decreasing response rate with increasing age of the infected individuals. The highest response rate was found in the case of the youngest age group while the lowest response rate was found among the subjects belonging to the older groups (56-65 years). Our results in the case of various age groups are in conformity with the previous studies [21, 19]. Although age dependent response rates are well documented locally, yet, the average response rate in the case of HCV 3a infection observed in this study is far lower than the local studies conducted earlier**.**

Conclusion

The efficacy of conventional IFN-based therapies is decreasing against HCV 3a infection in Khyber Pakhtunkhwa, Pakistan. Possible reasons for the phenomenon observed may be the rise of variant HCV 3a or low efficacy of recombinant IFN due to poor shipment and storage conditions.

Author's Contribution

IA designed the study and advised about the protocols. RU and NR carried out sampling and experimental procedures. SA, FZ ,RM, MA and ARS helped with experimental procedures and manuscript preparation. SA critically reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Corresponding Author:
Ijaz Ali, PhD
Head Division of Health Biotechnology
Institute of Biotechnology and Genetic Engineering Peshawar
The University of Agriculture Peshawar
0092-331-9378337

References:

1. Dash, S., A. B. Halim, H. Tsuji, N. Hiramatsu, and M. A. Gerber. 1997. Transfection of HepG2 cells with infectious hepatitis C virus genome. The American journal of pathology 151: 363.
2. Lok, A. S., L. B. Seeff, T. R. Morgan, A. M. Di Bisceglie, R. K. Sterling, T. M. Curto, G. T. Everson, K. L. Lindsay, W. M. Lee, and H. L. Bonkovsky. 2009. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology 136:138-148.
3. Idrees, M., and S. Riazuddin. 2008. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infectious Diseases 8:69.
4. McHutchison, J. G., S. C. Gordon, E. R. Schiff, M. L. Shiffman, W. M. Lee, V. K. Rustgi, Z. D. Goodman, M. H. Ling, S. Cort, and J. K. Albrecht. 1998. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. New England Journal of Medicine 339:1485-1492.
5. Poynard, T., P. Marcellin, S. S. Lee, C. Niederau, G. S. Minuk, G. Ideo, V. Bain, J. Heathcote, S. Zeuzem, and C. Trepo. 1998. Randomised trial of interferon [alpha] 2b plus ribavirin for 48 weeks or for 24 weeks versus interferon [alpha] 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. The Lancet 352:1426-1432.
6. Rehman, L. U., I. Ullah, I. Ali, I. A. Khan, A. Iqbal, S. Khan, S. H. Khan, K. U. Zaman, N. U. Khan, Z. A. Swati and A. T. Jahangiri. 2011. Active hepatitis C infection and HCV genotypes prevalent among the IDUs of Khyber Pakhtunkhwa. Virology Journal 8:327.
7. Moatter, T., A. Shah Hussainy, S. Hamid, Z. Ahmad, and S. Siddiqui. 2002. Comparative analysis of viral titers and histologic features of Pakistani patients infected with hepatitis C virus type 3. International journal of infectious diseases 6:272-276.

8. Khan, N. U., I. Ali, N. U. Ahmad, A. Iqbal, L. U. Rehman, I. Munir, M. U. Rehman, S. Khan, S. Ali, L. Siddique and Z. A Swati. 2011. Prevalence of active HCV infection among the blood donors of Khyber Pakhtunkwa and FATA region of Pakistan and evaluation of the screening tests for anti-HCV. Virology Journal 8:154.
9. Ali, A., H. Ahmad, I. Ali, S. Khan, G. Zaidi and M. Idrees. 2010. Prevalence of active hepatitis c virus infection in district mansehra pakistan. Virology Journal 7: 334.
10. Ahmad, B., S. Ali, I. Ali, S. Azam and S. Bashir. 2012. Response rates of standard interferon therapy in chronic HCV patients of Khyber Pakhtunkhwa (KPK). Virology Journal 9: 18.
11. Ali, S., I. Ali, S. Azam and B. Ahmad. 2011. Frequency distribution of HCV genotypes among chronic hepatitis C patients of Khyber Pakhtunkhwa. Virology Journal 8: 193.
12. Farid, A., M. A. Sherbiny, A. Osman, N. Mohamed, A. Saad, M. Shata, D. H. Lee, A. Prince, and G. Strickland. 2005. Schistosoma infection inhibits cellular immune responses to core HCV peptides. Parasite immunology 27: 189-196.
13. Zein, N. N. 2000. Clinical significance of hepatitis C virus genotypes. Clinical Microbiology Reviews 13:223.
14. Zein, N. N., J. Rakela, E. L. Krawitt, K. R. Reddy, T. Tominaga, and D. H. Persing. 1996. Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Annals of internal medicine 125:634-639.
15. Fried, M. W., M. L. Shiffman, K. R. Reddy, C. Smith, G. Marinos, F. L. Gonçales Jr, D. Häussinger, M. Diago, G. Carosi, and D. Dhumeaux. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. New England Journal of Medicine 347: 975-982.
16. Batool, U. and S. Qureshi. 2006. Declining sustained virological response in hepatitis C. Journal of the College of Physicians and Surgeons Pakistan 16: 187-91.
17. Farooqi, J. I., F. J. Rukhsana and H. Khan. 2002. Interferon alpha 2b monotheraphy and in combination with Ribavirin as initial treatment for chronic hapatitis C. Journal of the College of Physicians and Surgeons Pakistan 12: 82-5.
18. Khan, A. A. and S. Sarwar. 2009. Response to combination therapy in Hepatitis Virus C Genotype 2 and 3. Journal of the College of Physicians and Surgeons Pakistan 19:473.
19. Akram, M., M. Idrees, S. Zafar, A. Hussain, S. Butt, S. Afzal, I. U. Rehman, A. Liaqat, S. Saleem, M. Ali and A. Butt. 2011. Effects of Host and virus related factors on Interferon-a +ribavirin and Pegylated-interferon+ribavirin treatment outcomes in Chronic Hepatitis C patients. Virology Journal 8: 234.
20. Norris, F. H. 1992. Epidemiology of trauma: Frequency and impact of different potentially traumatic events on different demographic groups. Journal of consulting and clinical psychology. 60: 409.
21. Zuberi, B. F., F. F. Zuberi, S.A. Memon, M. H. Qureshi, S. Z. Ali and S. Afsar. 2008. Sustained virological response based on rapid virological response in genotype-3 chronic hepatitis C treated with standard interferon in the Pakistani population. World Journal of Gastroenterology 14: 2218–2221.

11/16/2013