RESEARCH ARTICLE

Virological Response to Conventional Interferon Therapy Combined with Ribavirin against Various HCV Genotypes in Khyber Pakhtunkhwa, Pakistan

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Abstract

Background: Response to antiviral therapy has been linked to different genotypes and this impacts on clinical management. Data on general responses to standard interferon (IFN) against HCV infection exists but little is known regarding HCV genotype specific responses. Purpose: Therefore, we attempted to determine genotype specific responses of chronic HCV patients, having different HCV genotypes, to standard IFN and ribavirin combination therapy administered for a period of six months in Kybher Pakhtunkhwa province of Pakistan. Materials and Methods: HCV genotype was determined for all serum samples. Each patient received standard IFN combination therapy with ribavirin administered at dose of 3 MIU three times a week and 800-1200 mg/ day, respectively, for a period of six months. After completion of this therapy, PCR was performed for all course completed subjects. Results: Out of total 51 selected patients the most abundant genotypes were 3a (49.0%) and 1a (21.6%) followed by 3b (9.8%), 1b (7.84%), 2a (7.84%) and untypable (3.94%). Moast responsive genotypes were 2a followed by 3a, with end of treatment responses of 77.7%, and 72.2%. Responses for 3b, 1b and untypable were 66.7%, 33.3% and 0%, respectively. Conclusions: IFN response is efficient in case of 2a and 3a genotypes while in case of untypable genotypes, further categorization is required to know about genomic sequences and to adopt some new regimes against these genotypes.

Keywords: HCV infection - genotypes - interferon - ribavarin - polymerase chain reaction - liver function tests

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Introduction

Hepatitis C is the infection caused by hepatitis C virus (HCV). Hepatitis C infection leads to liver cirrhosis and hepatocellular carcinoma and is an indication for liver transplantation in many countries (Wasaly, 2000. Shi, 2012). HCV has complex structure; its genome is 9.6 kb and encodes ten different proteins, playing its role in the structure as well as in replication of the virus. HCV has been classified into six main genotypes and more than 50 subtypes (Shi, 2012. Simmonds, 1999). Worldwide, genotype distribution varies like genotype 2 and 3 has worldwide distribution while genotype 1 is mostly prevalent in Europe (Zein et al, 1996).

The heterogeneity of HCV has great impact on the sensitivity and specificity of serological and virological assays for the detection of HCV infection. Little is known about the role of HCV in the progression of the disease and response to IFN therapy. HCV heterogeneity is helpful in tracing sources of HCV epidemics.

Different genotypes have different virologic response to IFN-based therapy. Like in genotype 1, Sustained Virologic Response (SVR) rates were almost half of that found in HCV genotype 2 or 3 infected patients (MuCHutchison et al, 1998). In genotypes 4, 5, and 6, the relative response rates to IFN therapy are less well defined, but their response is most probably similar to response found in genotype 1 rather than HCV genotypes 2 and 3. While in HCV subtypes (for example, HCV-1a and HCV-1b) the response rates are similar (Pawluotsky, 2002).

The aim of this study was to determine the efficacy of IFN alpha and Ribavirin combination therapy in chronic HCV patients of KPK. Nowadays the standard therapy is the Pegylated (Peg- IFN), but as the response rate of conventional therapy is almost the same as Peg-IFN, due to the prevalence of genotypes 2 and 3 in Pakistani

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population, therefore conventional therapy is considered as the choice of treatment in Pakistan (Iqbal, 2003).

Response of antiviral therapy has been linked to genotype and different genotypes have different clinical management (Zein et al, 1996). As data on general response of standard IFN against HCV infection exists but the data regarding HCV genotype specific response in KPK province of Pakistan is very rare. Therefore, we attempted to sort out genotype specific response to standard IFN and Ribavirin combination therapy administered for a period of six months.

Materials and Methods

Ethical Approval: Since the study population is human, therefore its ethical approval has been given by the Post Graduate Medical Institute Institutional Research and Ethics Board (PGMIIRE), reference number 1405.

To evaluate the response of standard IFN combination therapy among chronic HCV patients having different HCV genotypes, 51 subjects were selected randomly of different sex and locations. Blood samples were collected from all these patients with consent form. Serum was isolated from all samples and stored at -20 C0. These patients were already diagnosed as having viremia (active HCV infection).

HCV genotype was determined for all serum samples by following the method of Ohno.et.al. (Ohno, 1997). Each one of the patient, standard IFN combination therapy with Ribavirin was administered with a dose of 3 MIU (Million International Unit) thrice a week and 800-1200 mg/day, IFN and Ribavirin respectively for a period of six months. Biochemical profile like LFTs (Liver function tests) and CBCs (complete blood count) was determined before, during and after the therapy. After completion of six month therapy, PCR (Polymerase Chain Reaction) was performed for all course completed subjects.

Results

As different genotypes have difference in clinical management, therefore we conducted this study to find out response of different genotypes to six months of standard IFN combination therapy. In this regards a total of 51 patients who had active HCV infection, were selected randomly for genotype specific response.

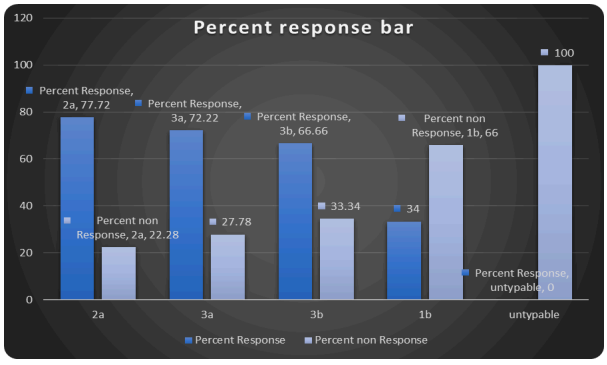

Figure 1. Percent Response Bar of Different HCV Genotypes

Table 1. Percent Frequency of Different HCV Genotypes among Chronic HCV Patients

Genotypes	Isolates	Total numbers of isolates	Frequency %
3	3a	25	49.01
	3b	5	9.8
1	1a	11	21.58
	1b	4	7.84
2	2a	4	7.84
Untypable		2	3.92
Total		51	100

Before starting therapy, genotype was determined for each patient by type specific PCR method as discussed above. Combination therapy was given to all patients for equal duration and equal dose of IFN and Ribavirin. After completion of therapy and performing PCR for all course completed subjects, the results obtained were as. Out of total 51 selected patients the abundant genotypes were 3a (49.01%) and 1a (21.58%) followed by 3b (9.80%), 1b (7.84%), 2a (7.84) and untypable (3.94%) (table.1). Percent response among these different genotypes was noted as, in case of 2a, the response was 77.72%, while in case of 3a it was 72.22%. Response in other genotypes like 3b, 1b and untypable was 66.66%, 33.33% and 0% respectively (Figure 1).

Discussion

Infection with any HCV genotype irrespective of the level of viremia can lead to cirrhosis, end-stage liver disease, and hepatocellular carcinoma. Such complications frequency is almost similar with each genotype. The role of HCV genotypes in the progression of liver disease is one of the most controversial areas of HCV research. It has been investigated by Amoroso et al. (Amoroso et al, 1998), that in patients having HCV genotype 1b, 92% of patients were converted into chronic infection compared with 33% to 50% in patients having other genotypes. This suggests that genotypes play an important role in chronicity of infection.

To find out genotype specific response, we selected 51 patients, randomly from KPK population having chronic HCV infection. HCV genotyping was done using type specific PCR method and enrolled all patients for six months combination therapy.

The present study indicated that frequency of HCV genotype 3a was higher followed by 1a and 3b. The lowest frequencies were that of genotypes 1b, 2a and untypable among chronic HCV infected patients [Table 1]. The highest ETR (77%) to standard combination therapy was observed in case of HCV genotype 2a patients, followed by 3a (72.27%) and 3b (66.66%). The lowest response was observed in case of HCV genotype 1b (33.33%), while null response was shown by patients having untypable HCV genotypes [Figure 1].

Different geographical regions have different HCV isolates distribution, like HCV genotypes 2 and 3 have worldwide distribution (Zein et al, 1996). But in KPK, according to our study the most abundant genotypes were

3a followed by 1a [Table.1]. This is almost an agreement with the previous study conducted in Pakistan and KPK regarding frequency distribution of HCV genotypes (Latif, 2011. Idress, 2008). The lowest frequency among chronic HCV patients was that of 1b, 2a and untypable [Table 1]. The distribution of genotype 1b is mostly in European states (Puro, 1995) but lower in Pakistan and also in KPK population (Latif etal, 2011). While untypable distribution was the lowest one and it has also been shown by a study conducted in KPK that untypable genotypes are present in chronic HCV patients (Latif et al, 2011).

According to this study, combination therapy was more effective in case of chronic HCV patients with genotype 2 as compared to genotype 3 [Fig 1]. Other studies conducted in Pakistan have earlier revealed higher response rates (80-85%) in case of HCV 2a and HCV 3a, as these are predominant in Pakistan (Sarwar, 2006). In Asians, approximately 90% response rate among HCV 2/3 patients has been found (Yu, 2009). Comparatively higher response of HCV genotype 2 might be attributed to the responsive nature of this genotype. It was assumed that HCV genotype 2 and 3 require similar treatment duration. But evidence now indicates that this might not be the case. As patients having HCV genotype 2 seems to show better response to IFN–based therapy and consistently having higher SVR rate as compared to type 3, 80-93% compared with 66-80% respectively, when treated for 24 weeks (Alessandra, 2010).

The response of other genotypes was comparatively low or null that is genotype 1b, has 50 % response while untypable genotypes had no response to conventional combination IFN therapy [Fig 1]. The response rate in Asian patients having genotype 1 has been found to be 70% (Zein et al, 1996). As genotype 1 is not prevalent in Pakistani population, mostly found in European states and it has been reported that response of genotype 1 and 4 to IFN therapy is 60-70% and may require longer duration of IFN-therapy (Poynard et al, 1998). Moreover the presence of HCV genotype 1 or 4 together has been considered as the strongest baseline predictor of non-response to treatment (Neumann et al, 2000). Beside this it has been suggested that mutations in the HCV nonstructural (NS) 5A protein, considered as the IFN sensitivity region might be the cause of resistance to anti-viral therapy in case of HCV-1– infected individuals. Moreover it has been suggested by Viral kinetic studies that turnover of hepatocytes in case of HCV genotype 1 infected patients is slower than other genotypes after initiation of anti-viral therapy (Zeuzem et al, 2001). Hence there is need of more aggressive and longer duration of treatment regimens (Buti, 2003. Berg et al, 2003) having capability of continuous stress and pressure over the virus. While the null response in case of untypable might reflect some new isolates/quasispecies evolution. The evolution of viral quasi-species is another element regarding resistance to anti-viral therapy (Okara, et al, 1992). As most of the time quasi-species complexity has been observed in non-responders than that of patients having undetectable HCV RNA after six months of treatment completion (Moribe et al, 1995). Due to migration of different cast, community and region people to this province the ratio of finding untypable genotype is becoming more .These genotypes should be explored and some serious attention must be made to investigate these nonresponsive genotypes. Hence it is proved that in KPK the abundant genotypes are 3a and 1a, while responsive genotypes are 2 and 3.

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