ORIGINAL ARTICLE

ASSOCIATION OF CLINICAL PARAMETERS AND INTERFERON ALPHA/BETA RECEPTOR SUBUNIT 2 IN HCV PATIENTS RESISTANT TO INTERFERON THERAPY

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Background: Interferon therapy is an expensive treatment for hepatitis C virus (HCV) which may results in undesirable side effects. About 50% of chronic HCV patients do not respond to interferon (IFN) therapy for unknown reasons. It is important to determine the association of clinical parameters with IFNAR-2 in HCV patients, resistant to IFN therapy. **Methods:** HCV genotype 3 patients were included in study (n=20, between 30–60 age group) and categorized into two groups, the experimental group including patients responding to interferon treatment and the control group consisting of interferon resistant patients. Blood samples and Liver biopsy specimens were collected from all HCV patients. Clinical parameters included in the study were (i) pre-biopsy tests (platelet count, bleeding time, prothrombin time) (ii) HCV viral genotype 3 (iii) HCV-RNA and (iv) liver functions (ALT, ALP, and bilirubin). Molecular investigations were carried out by using PCR and gel electrophoresis to identify IFNAR-2 in all HCV genotype 3 patients. **Results:** Experimental results from clinical testing of non-responder (resistant) group, showed significantly increased ALT level (64.13±17.48 U/L) as compared to responder group (46±6.3 U/L). Hemoglobin (Hb) level was also found significantly increased in non-responder group (12.9±1.8 g/dL) in comparison with responder group (10.3±0.48 g/dL). Further molecular testing revealed that all interferon resistant patients showed the presence of IFNAR-2. **Conclusion:** Experimental observations revealed that non-responder HCV genotype 3 patients showed elevated ALT and Hb levels and presence of IFNAR-2 protein. Therefore, experimental results of this study signify the importance of monitoring clinical parameters in parallel with IFN therapy in non-responder HCV patients.
Keywords: HCV, Genotype, IFN therapy, Liver Function tests

INTRODUCTION

Almost 15–25% of HCV infections clear by themselves, whereas 60–80% cases progress towards chronic infectious stage which is marked as persistence of HCV-RNA, six months after acute infection.[1] Multiple factors which cause the disease to become chronic include, age, gender, immune response and race. Cirrhosis and hepatocellular carcinoma (HCC) also develop in chronic HCV patients. Rate of progression towards chronic HCV stage is low between 12–25 years age group[2], particularly in young women, whereas risk of development of hepatocellular carcinoma is two to four times higher in Asian and African American infected population.[3,4]

HCV infection at acute phase generally shows symptoms like fever, nausea or abdominal pain commonly in 20% patients. There is a chance of jaundice after 3–12 weeks of infection or after exposure. Generally, it has been observed that onset of jaundice in acute phase of HCV, favours immunity and lowers the risk of development of chronic stage. Clinical investigations showed raised levels of ALT (103 to 107) within 1–2 weeks after exposure and at this stage viral RNA is detectable in serum.[5,6,7] Twenty-five to thirty percent patients suffer from most chronic stage that is cirrhosis, which develops approximately in two decades.[8] Normally, it may take 24–27 years for HCV patient to develop HCC.[9] Interferon-α/β (IFN-α/β) is a part of Host's immune response which resists intracellular pathogenic infections by involving innate and adaptive immune systems.[10] Interferon-α treatment improves liver function, which result in reduced ALT levels, low viral load and reduction in HCV progression. Still it is not clear that IFN-α treatment destroys the HCV infected cells or not.[11,12] Regarding response to the treatment it has been observed that it is variable among type of HCV genotypes, some are more responsive and others not.[13] Duration of treatment also depends upon the type of HCV genotype, such as genotype 1 and 4 require 48 weeks of treatment whereas genotype 2 and 3 need only 24 weeks of treatment.[14] Clinically successful treatment marked by sustained viral response (SVR) is obtained after 24 weeks post treatment, at this stage viral RNA is no more detectable in serum, sometime this viral response may be early or rapid.[15]

Interferon-α treatment mechanism starts with signaling pathway, in which IFN binds with IFNA-1 and 2 receptors. Interferon alpha and beta receptor subunit 2 (IFNAR-2) has 1,000 times more affinity for IFN as compared to interferon alpha and beta receptor subunit 1 (IFNAR-1). Both receptors form dimers upon

binding with IFN. Dimerization results in phosphorylation of tyrosine residue by involving Janus Kinases (JaK). This orientation provides downstream signaling for transcription proteins (STAT), which results in transcription of interferon regulated genes and respective proteins. These proteins attack the viral RNAs and block their translation and further hinder viral replication.[14] Research revealed that HCV has potential to inhibit the IFN signaling pathway and can avoid IFN's antiviral immune system of the host.[16] There are various associated host (patient) and viral factors which affect the success of IFN treatment, such as age, gender, body mass index (BMI), cirrhosis, diabetes, ethnicity, alcohol intake, drugs and steatosis.[14,17,18]

Therefore, for successful IFN treatment and HCV eradication, it is important to uncover the HCV-IFN-α escape mechanism, which may deal with inhibitory role or lack of IFNAR-2 expression, to explain the HCV resistance for IFN treatment. This study was designed to investigate the association of clinical parameters and IFNAR-2 in HCV patients resistant to IFN treatment.

MATERIAL AND METHODS

This cross-sectional analytical study was conducted at the Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi in collaboration with Department of Medicine, Holy Family Hospital, and Military Hospital, Rawalpindi. Research protocol was approved by Ethical Review Committee, Army Medical College, Rawalpindi. Written informed consent in accordance with declaration of Helsinki was obtained from all the patients involved in the study.

Twenty HCV patients, aged 30–60 years, were recruited for this study and divided into two groups; group 1 (responder to IFN treatment, n=5) and group 2 (non-responder to IFN treatment, n=15). HCV-RNA positive patients for genotype 3, moderate liver histopathology, IFN therapy response, and patients who had at least 1.5-fold increase in their liver enzymes levels were included. Patients above 60 years and those with autoimmune disorders were excluded from analysis. The history was taken and documented by the concerned medical specialist. Blood samples were collected from group 1 (responder to IFN treatment).

After collection blood samples were processed and preserved for further analysis. Liver biopsy specimens were collected from HCV patients, group 2 (non-responder to IFN treatment). Liver biopsy samples were transported, while preserved in liquid nitrogen and stored (-80 °C) for further use. Clinical factors of subjects, included in study were (i) pre-biopsy tests, such as bleeding time, prothrombin time, platelet count, (ii) liver function tests, i.e., ALP, ALT, Bilirubin, (iii) HCV genotyping, and (iv) viral-RNA load.[19]

The primers were designed by using primer3 plus web tool for human interferon receptor 2 (IFNAR-2) (NCBI Reference Sequence: NM_207585.1). F1 IFNAR2: 5´ACAAGTG GCGG TGGCTATAC3´, R1 IFNAR2: 5´TCAGGATCCTCTGGGTCAAC3´. RNA was isolated from liver tissue biopsy and blood sample by using GeneJET™ RNA Purification Kit (Fermentas). cDNA was synthesized by using RevertAid Premium first strand cDNA Synthesis Kit (Fermentas). cDNA was further subjected for PCR amplification. The PCR conditions were 95 °C-6 min; 94 °C-45 sec; 54.5 °C-45 sec; 72 °C-1.3 min for total 35 cycles and final extension at 72 °C-10 min.

The PCR products of IFNAR-2 cDNA were visualized by running the products on agarose gel (2%) and imaged under UV transilluminator. Further gel images were quantified for intensity profile of bands through GelQuaNET.lnk software program.[20] Results were presented as Mean±SD. Statistical evaluation was conducted by using student's t-test and ANOVA, and p≤0.05 was taken as statistically significant.

RESULTS

There were 11 (55%) males and 9 (45%) female patients in the study with a mean age of 39.84±11.12 years. Laboratory investigations for clinical parameters such as liver function tests (ALT, Bilirubin and ALP), Hb (g/dL), WBCs and Platelets (PLTs) counts were analyzed in both groups and results were presented in Table-1. Experimental data showed that plasma bilirubin level had no significant change between responder and non-responder groups. ALT level was found significantly increased in non-responder group as compared to responder, whereas no significant change in ALP level observed between these groups.

Table-1: Comparison of study variables between responders and non-responders

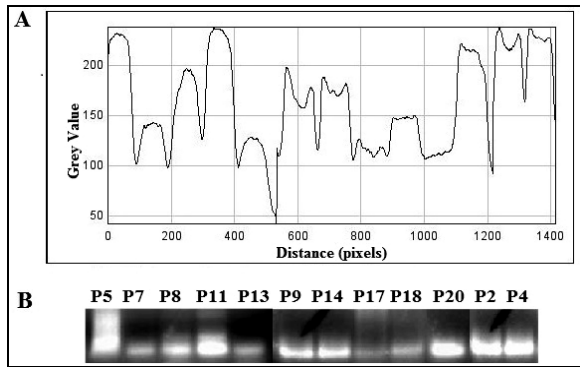| Parameter/Groups	Clinical parameters					
	ALT	Bilirubin	ALP	Hb	WBCs	PLTs
Normal Range	(Up to 42 U/L)	(0–1 mg/dL)	(65–304 U/L)	(12–16 g/dL)	$(4–11×10^3/mm^3)$	$(150–400×10^3/mm^3)$
Non-responder	64.13±17.48	1.3±0.36	215.2±4.1	12.9±8	7533.3±5.4	224.1±17.4
Responder	46±6.3	1.28±0.45	198.4±5.6	10.3±0.5	6800±4.3	243.2±18.4
p	0.003*	0.90	0.67	0.0001*	0.37	0.60

All values are Mean±SD, *Significant

Figure-1: (A) An intensity profile of IFNA-2 receptor's cDNA. (B) Detection of IFNA-2 receptor's cDNA in liver tissue biopsy of HCV patients (n=13)

Hb levels were also increased significantly in non-responder group. It has also been observed that, WBCs and PLT count remain increased in non-responding group but results are insignificant. Furthermore, liver biopsies were taken from resistant patients and processed for IFNAR-2 RNA detection. Figure 1 shows presence of IFNAR-2 RNA (cDNA) in all non-responder patients. Intensity profile varies from patient to patient, depending upon the stage of HCV progress in respective patients.

DISCUSSION

In this study we focused on association of clinical parameters and identification of IFNA-2 receptor in HCV genotype 3 patients resistant to IFN therapy. Our study found that mean plasma ALT levels decreased significantly in responder group, whereas non responder group showed increased level, which is supported by another study that also showed reduced plasma ALT levels in responder group (within 10 weeks of therapy when compared with non-responders and relapse-cirrhotic patients).[20]

It is known that IFN-α immune response develops cellular resistance for HCV infection at the virus's entry or/and replication stage, while the response to therapy is determined by expression of IFN-α receptors.[19] IFN resistance occurs due to various host and viral factors. One important host factor is IFNAR-2 which plays a crucial role in the activation of anti-viral genes. Total or partial failure of IFNAR-2 expression, results in the malfunctioning of the Interferon signalling pathway. Our results from gel electrophoresis also showed varied degrees of band intensities of IFNAR-2 RNA expression. Previous study described that its expression has been decreased by HCV E2, C (structural proteins) and NS5A (non-structural protein). It reduces the responsiveness to the treatment with IFN and causes an acceleration in viral replication leading to the inflammation and liver fibrosis in HCV patients.21 IFNAR-2 detection in liver

may uncover the cellular factors involved in interferon resistance. It will also help to determine IFN treatment response in HCV patients.[22]

The response to interferon therapy may be linked to the concentration and expression of IFNAR-2 on the cell surface.[23] Expression level of IFNAR-2 in liver will also be helpful to evaluate the IFN treatment response in chronic patients.[22] Furthermore in PBMCs, IFNAR-2 expression can be helpful to monitor its intrahepatic cellular expression.[24] Our study was designed to investigate the presence of IFNAR-2 in IFN resistant HCV patients. Results showed the presence of IFNAR-2 expression in 80% of interferon resistant patients, i.e., non-responders to IFN therapy, while all the responders were also found IFNAR-2 positive. There is considerable uncertainty regarding the expression of IFNAR-2 in HCV patients resistant to IFN therapy. As few studies show that IFN therapy response in chronic HCV patients is associated with the expression of IFNAR-2.[25] Taniguchi et al[26] determined the higher level of expression of IFNAR-1 and a higher ratio of IFNAR-1 to IFNAR-2 in patients who had SVR after IFN therapy.

Our results were strengthened by previous study, which established that IFNAR-2 expression was detected in Huh-7 cell, resistant to IFN treatment.[27] Our study revealed that there was no significant difference observed in the presence of IFNAR-2 expression in HCV patients both groups (responders and non-responders). We conducted this study with limited number of samples due to less availability of samples from patients of HCV genotype 3. Based on these results it is suggested that there is need to investigate further, including larger sample size, ranging from different ethnic groups, with genetic variation and geographical distribution.

CONCLUSION

Non-responder HCV genotype 3 patients showed elevated ALT and Hb levels along with presence of IFNAR-2. This study highlights the importance of monitoring the clinical parameters in parallel with IFN therapy in non-responder HCV genotype 3 patients. Identification of IFNAR-2 RNA or intensity profile can be next point of investigation as it may provide the information about the treatment potential or HCV progression rate.

CONFLICT OF INTEREST

There was no conflict of interest.

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