DETECTION OF STAT-1 IN BLOOD SPECIMENS OF INTERFERON RESISTANT HEPATITIS C PATIENTS

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ABSTRACT

Objective: To evaluate the expression of STAT-1 (Signal Transducers and Activators of Transcription-1) in HCV patients non responder to interferon treatment.

Study Design: Case control study.

Place and Duration of Study: The research was carried out at Army Medical College Rawalpindi from January to July, 2012.

Patients and Methods: The study after approved by institute's ethics committee was conducted on 15 HCV infected patients who were non responder to interferon therapy and 5 controls responder to interferon therapy. Their age, sex, body mass index (BMI) and marital status was noted. PCR based detection of STAT-1 mRNA was carried out in blood of HCV infected patients resistant to interferon therapy as well as controls. Data was presented in the form of frequencies and percentages and *p* values were calculated using Fisher exact test and student t-test.

Results: Results showed that more males were resistant to interferon therapy as compared to females. The mean age was less in responders as compared to non responders. Forty percent of the HCV infected patients non responder to interferon therapy were positive for STAT-1 expression.

Conclusion: STAT-1 blood expression can predict treatment response in HCV patients undergoing interferon treatment.

Keywords: Blood expression, HCV, Interferon resistance, Predictive factor, STAT-1.

INTRODUCTION

Hepatitis C Virus (HCV) has victimized 180 million people around the world[1] and about 10 million people in Pakistan[2]. HCV causes acute and chronic hepatitis which results in liver damage that may lead to cirrhosis and hepatocellular carcinoma[2]. HCV, a positive sense single-stranded RNA with 9.6 kb size, is an enveloped virus belonging to family flaviridae having six genotypes with various subtypes and works to some extent for its survival by limiting the interferon-signaling pathway[3]. HCV has varied geographic distribution within Pakistan among the high prevalent countries[4]. HCV control and prevention depends on global distribution of the virus, determination of the risk factors involved, and assessment of the accelerating factors responsible for progression of the disease [4]

Interferon alpha (α) and beta (β) are subtypes of type I interferon (IFN)[5]. Interferon-α in combination with ribavirin is standard HCV treatment and through the next decade, the pegylated interferon-α will remain as basic treatment for HCV as new interferon formulations are in premature stages of development[6]. Interferon-α/β resists intracellular pathogens and alarms immune response cells. Interferon-α is being used for treatment of Hepatitis B Virus (HBV) and HCV infections[7].

In order to understand the role of cellular factors that are important in interferon signaling cascade in blood of interferon resistance, a study was designed to detect STAT-1, an important cellular factor, in interferon resistant HCV patients.

MATERIAL AND METHODS

Research was case control study, carried out at Army Medical College Rawalpindi from

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January to July, 2012. The study was approved by institute's ethics committee. The study included patients infected with HCV resistant to twice course of interferon treatment (n=15) and those subjects (n=5) as control that were previously infected with HCV but responded to interferon treatment. The patients who refused to take part in the study were not included in the final data. The subjects included in the study were selected based on convenience sampling method. Patients were explained about the study's significance. Written and informed consent was obtained from the patients. Their age, sex, BMI and marital status was noted. Blood was collected from these patients under strict aseptic measures and processed for RNA extraction same day. RNA was extracted from blood using, Fermentas GeneJET™ RNA Purification Kit and total RNA was isolated, quantified and 1µg was used further for cDNA synthesis using RevertAid Premium First Strand DNA, Fermentas. Based on previously available sequence on NCBI, primers specific to STAT-1 was designed by using different bioinformatics tools i.e., Primer 3, E-PCR and Oligo calc. The primers used were forward primer 5'GTCGG GGAATA TTCA-GA GCA 3' and reverseprimer 5'TGATC-ACTCTT-TGCCAC-ACC3'. PCR based detection of STAT-1 mRNA was carried out in blood of HCV infected patients resistant to interferon therapy as well as controls. PCR amplification was performed with 25 µl reaction mixture in 0.2ml microtube. The reaction mixture contained 5µl cDNA as template, 0.3 µl (20 µM) of each forward and reverse primer, 2.5 µl 10X PCR buffer, 1.5 µl MgCl2 (25 mM), 0.5 µl dNTPs (0.25 mM of each dNTPs, 0.2 µl Taq DNA polymerase (0.5 unit) and 18.7 µl PCR water. The thermocycling conditions were: initial 5 min denaturation of template DNA at 95°C as first step followed by 33 cycles of 95°C for 30 sec (denturation), 51°C for 30 sec (annealing), and 72°C for 30 sec (polymerization), followed by a final extension of 10 min at 72°C in thermocycler. Data was presented in the form of frequencies and pe-rcentages as calculated using Microsoft Excel 2007 and p values were calculated using Fisher exact test and student T-Test using web-based tool (http://www.socscistatistics.com/tests/chisquare/default2.aspx).

Table-I: Characteristics of the subjects included in the study.

Characteristics of subjects		Resistant to Interferon therapy (n=15)	Responders (n=5)	*p*-value
Age		44.1 ± 7.9	37.2 ± 7.1	0.10
Sex	Male	10 (66.67%)	3 (60%)	1
	Female	5 (33.33%)	2 (40%)	
Marital Status	Single	7 (46.67%)	None	0.114
	Married	8 (53.33%)	5 (100%)	
BMI (body mass index)		23.0 ± 1.2	22.2 ± 0.4	0.167

RESULTS

The study included 15 HCV infected patients non-responders to interferon treatment and 5 previously infected HCV subject responder to interferon treatment. The difference in mean age, BMI and sex ratio was not significant among the two groups. Results showed that most of the HCV infected patients resistant to standard interferon treatment (i.e., 60%) didn't express the STAT-1 in their blood

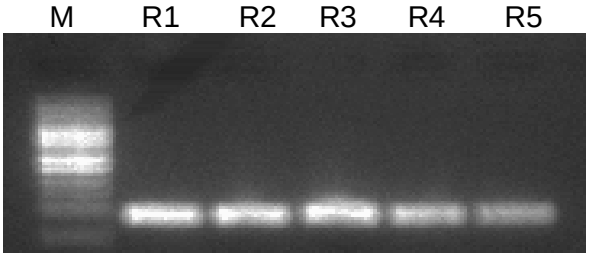

Figure-1: HCV Patients Responders to Interferon Therapy: PCR based results showing expression of STAT-1 in HCV patients who responded to the standard interferon therapy showing bands ~200 bp length.

whereas all responders expressed STAT-1 (as shown in table 1). The representative results of PCR based detection of STAT-1 in blood of subjects included in the study are shown in the fig-1 and 2.

Expression of STAT-1 in HCV patients who responded to the standard interferon therapy showing bands ~200 bp length.

PCR based results showing expression of STAT-1 in HCV patients who did not respond to the standard interferon therapy but were positive for STAT-1 expression; showing bands in ~200 bp length.

DISCUSSION

HCV has reached epidemic proportion in our country with great variation in its geographical distribution. It is proposed that IFN are a family of cytokines that play a very important role in innate immunity and protects humans from infections against number of viruses including HCV and other intracellular organisms[8].

STATs are important for activation of genes playing antiviral role. Studies revealed that STATs perform different vital functions in the liver including its participation in antiviral immune defense, development of inflammation, apoptosis and antitumor responses if they are not expressed or if expressed in insufficient quantities, this may hinder interferon stimulated intracellular cascade promoting replication and persistence of Hepatitis C Virus[9].

The recommended therapy for HCV is Peg-Interferon–α 2b or Peg-Interferon–α 2a in combination with ribavirin has 40–50% success rate in HCV genotypes 1/ 4 and 75–90% in HCV genotypes 2/3[1]. Identifying the factors responsible for interferon resistance, can be useful to predict the therapy response. Viral, cellular and other factors (molecular mechanisms induced by HCV proteins to inhibit interferon signaling flow) can resist therapy response[3]. In viral factors, viral load, genetic diversity and specially the genotyping of the virus are important. It has been shown that in the same genotype group, variants share at least 70 percent of sequence homology and 30% showed appearance of quasi species. This could be another reason for reduced expression of STATs in HCV patients as these viral variants generated during the replication process, showing a different sensibility to the treatment. In Pakistan Genotype 3 is prevalent and was found to be present in 79.43% of the HCV patients. A proportion of patients have their genotype reported un-typeable. Presence of un-typeable samples indicated that some novel genotypes might be present in Pakistan, yet to be detected. Another proportion of patients resistant to treatment found to be infected with a mixed variety of genotypes[10].

Most of the patients included in the study were found to be accidently diagnosed as patients of hepatitis C virus infection with no clinical symptoms at all. This raised the possibility that their early viral load was >600,000 UI/ml which makes them less sensitive to the standard treatment. Compliance to the treatment for 24-48 weeks was another problem faced by the treating physicians. High cost of treatment was one of the main reasons for this non-compliance. Appearance of side-effects of the treatment especially loss of appetite and depression makes observance to the treatment difficult. The production of anti-IFN antibodies may also account for some of the failures of IFN therapy as they are directed against receptors-binding domain of IFN[11].

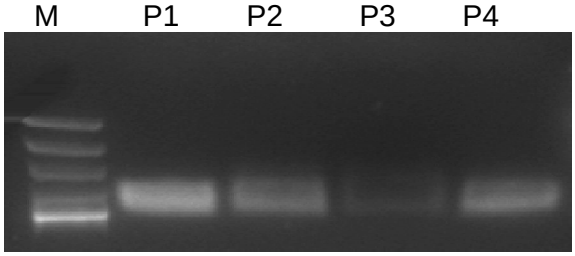

Figure-2: HCV Patients Resistant to Interferon Therapy: PCR based results showing expression of STAT-1 in HCV patients who did not respond to the standard interferon therapy but were positive for STAT-1 expression; showing bands in ~200 bp length.

Viral and host factors and the molecular mechanisms induced by HCV proteins can be associated with interferon resistance in hepatitis C patients[3]. It is therefore recommended that an animal or cell culture system more closely

resembling natural HCV infection should be designed and studied. In addition other genetic and molecular markers which are sensitive and feasible should be identified to improve the treatment plan of HCV patients.

The study also suggests that clinicians while planning anti-viral therapy for their HCV patients need to analyze the response probability for their patients through expression of STAT-1 which if detected have positive effect on the outcome of IFN therapy. Whereas in cases STAT-1 expression is not detected, another combination therapy should be explored and given as trial to avoid the longer duration, side effects and high cost of standard IFN therapy. It is also suggested that a broader level study should be conducted, focusing on both viral and host factors in the same patient so that the proportion of STAT-1 responsible for interferon resistance can be evaluated.

CONCLUSION

The finding indicated that expression of STAT-1 is an important factor in generating response of the host to HCV (interferon) therapy. It also showed the possibility that factors other than STAT-1 might also be involved in those resistant cases (40%) that showed expression of STAT-1.The lack of expression of STAT-1 does not seem to be the major cause of interferon resistance in HCV patients receiving interferon therapy.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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