DETECTION OF STAT-1 IN PATIENTS CO-INFECTED WITH HEPATITIS B AND C RESISTANT TO INTERFERON

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ABSTRACT

Objective: Current study was designed to determine the STAT-1 in co-infected patients of hepatitis B and C resistant to interferon therapy.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Biochemistry & Molecular Biology & Gastroenterology departments of various hospitals of Rawalpindi.

Material and Methods: The study included 15 co-infected patients of hepatitis B and C resistant to interferon therapy and 15 healthy individuals as control.

Methodology: Detection of STAT-1 was done by conventional PCR technique.

Results: Sixty seven percent of the patients were expressing STAT-1 in their blood while 33% of the patients did not have STAT-1. Controls showed 57% detection of STAT-1 and 43% did not exhibit STAT-1. Mean age of the patients and controls was 35.90 ± 8.95.Comparison between patients and controls was done by chi square test. Fisher exact probability value obtained was 0.287 which was not significant.

Conclusion: Patients suffering from hepatitis B and C co-infection resistant to interferon therapy revealed higher detection of STAT-1 which indicate greater liver damage, fibrosis and an extensive and severer disease course in co-infection.

Keywords: Co-infection, HBV, HCV, Interferon, STAT-1.

INTRODUCTION

Hepatitis B and C co-infection is very common in countries where chronic liver disease is caused by hepatotropic viruses. Signal transducer and activator of transcription (STAT-1) plays an important role in molecular signaling pathway. As HBV and HCV share common routes of transmission therefore their co-infection is not uncommon[1].

HCV may be present in blood either in free form, or in association with immunoglobulin or with low, high or very low density lipoproteins[2]. After intracellular localization HCV assembles itself[3]. HBV belongs to a family of small enveloped partially double stranded DNA viruses called Hepadnaviridae. They have a circular DNA genome and have a narrow host range[4]. It has an outer membrane which incorporates lipids and viral proteins[5]. Pegylated interferon is now considered a better treatment option than conventional treatment of hepatitis B and C. Interferon is antiviral cytokine produced by many cell types which help to protect against infection. They are released in response to inflammation and they help in survival of hepatocytes. As the magnitude of problem of co-infection is greater therefore this study has been designed to detect STAT-1 in blood of patients co-infected with hepatitis B and C to measure the influence of absence of STAT-1 in patients having interferon resistance. Co-infection of HBV and HCV is not uncommon especially in areas where these two viruses are endemic particularly in individuals with parentral infections[6]. Approximately 3-18% of patients having HBV also develop HCV. These patients face a severe course of liver disease, and a poorer survival rate[1]. Due to co-infection patients ultimately land into extensive necrosis, cirrhosis of liver and finally hepatocellular carcinoma[7].

Studies have shown that patients having co-infection with hepatitis B and C face a

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greater mortality risk as compared to healthy population. The mortality rates for HBV, HCV and HBV-HCV co-infection are 3.2%, 5.3% and 7.1% respectively[7]. Death rate is high due to continued drug use as well as due to complications. One of the factors responsible for hepatocarcinogenesis is co-infection with hepatitis B and C as HBV enhances HCV replication because both viruses are frequently isolated from liver biopsy samples of patients of cirrhosis and hepatocellular carcinoma. A study conducted by T. Stroffolini et al in Italy revealed that co-infection of HCV may occur alone or with HBV but 100% cases of delta infection occur with HCV. HBV infection was dormant but HCV was replicating. This important finding may be due to direct inhibition of HBV replication by HCV[6].Inhibition of HCV replication by drugs can alter the situation and recurrence of HBV replication takes place. This study emphasizes on giving high doses of interferon so as to inhibit both viruses replication[8]. In a study conducted by Khattaba et al showed an inverse relationship between HBV and HCV viral load in serum. HBV levels decreased when HCV levels increased and vice versa. This states in vivo interference of both viruses. There may be mutations in S gene of HBV or in core protein of HCV which may also affect suppressive activity of viruses on each other[9]. STATs are latent cytoplasmic transcription factors that become activated after recruitment to an activated receptor complex. They are total seven in number. They are STAT-1, STAT 2, STAT3, STAT 4, STAT5a, STAT5b and STAT 6. STAT proteins exert active role in antiviral battle against hepatitis as well as limiting injury, healing, inflammation and tumorigenesis. Progression of liver disease can be controlled by a number of cellular mediators among which JAK-STAT pathway plays a significant role. Up till now four JAKs have been identified JAK1-3 and Tyk 2. JAK-STAT signalling facilitates intracellular communication. JAK-STAT pathway was first identified in 1990s. It gets activated by almost 40 cytokines and growth factors[10]. Generally on binding with the receptor cytokines induce receptor dimerization and then JAK dimerization. After this

Table-1: Cross tabulation comparison of patient & control.

		Group		Total	Fisher Exact Test*p*-value
		Patient	Control		
	Positive	10	9	19	.999 ± 1
	Negative	5	6	11	
Total		15	15	30	

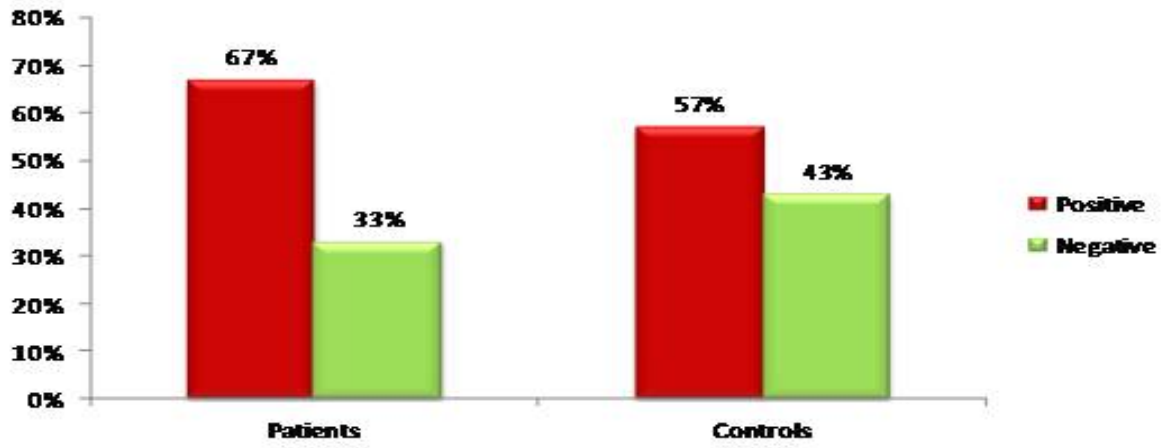

Figure-1: Frequency of expression of STAT-1 among patients and controls.

phosphorylation take place. JAK-receptor complex employs various STAT proteins and phosphorylates them. These phosphorylated STATs then form homodimers or heterodimers and translocates into nucleus[11]. Here they act as transcription factors and induce transcription of genes to regulate various cellular functions. STAT-1 enhances host defense and IFN-α treatment induced liver regeneration[12]. After binding of interferon[1] a signalling cascade begins and activate transcription of interferon stimulated genes[10]. There are three families of proteins which terminates the actions of STAT proteins Suppressor of cytokine signaling (SOCs), SH2 containing phosphatases (SHPs) and Protein inhibitors of activated STATs (PIASs). SOCs 1 inhibit STAT-1 with preferential inhibition of IFN-γ signalling. SOCs 3 inhibit IL-6 signalling in liver[12].Treatment with interferon is quiet effective but the role of immune response as well as viral factors play an important part in either its success or failure. Many studies have indicated that interferon α activates natural killer cells which mediates eradication of HCV infected hepatocytes. Interferon γ also activate STAT-1. Effective treatment of hepatitis Band C result in reduced complications and improve survival[13]. The principles that guide us regarding treatment of mono infection should be followed to treat co-infection also after taking into consideration which virus divides in patient with serological markers of HBV-HCV co-infection[14]. Pegylated interferon and ribavirin is considered to be the gold standard for treating co-infection[15]. Interferon in combination with ribavirin is given for 24 or 48 weeks to eradicate the infection[12]. Treatment of co-infection should be individualized based on patients variables such as hepatitis blood test results such as ELISA and PCR, DNA or RNA levels, patients prior exposure to antiviral treatment and the presence of other similarly transmitted diseases such as HIV.

Figure-2: Electropherogram of an ethidium bromide stained 2% agarose gel showing 200bp band of STAT-1 obtained with primer STAT-1 for HCV/HBV co-infected patients resistant to interferon therapy.

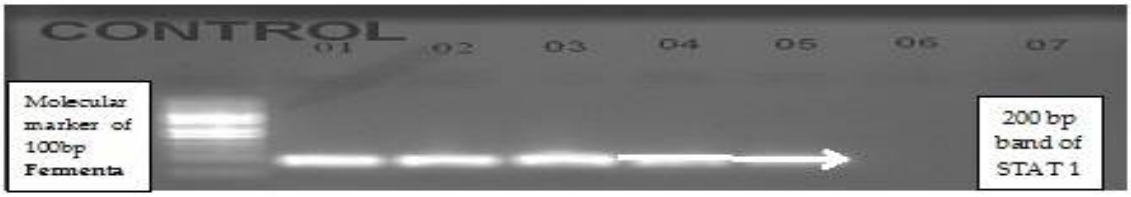

Figure- 3: Electropherogram of an ethidium bromide stained 2% agarose gel showing band pattern obtained with primer STAT-1 for healthy individuals.

MATERIAL AND METHODS

This cross-sectional analytical study was carried out at Department of Biochemistry & Molecular Biology & Department of Gastroenterology, Rawalpindi, over one year. Inclusion criteria was to incude only co-infected patients of hepatitis B and C. Exclusion criteria was to rule out presence of HBV alone, HCV alone, diabetes and hypertension. Blood samples of fifteen seropositive, co-infected patients of hepatitis B and C were collected from Departments of Gastroenterology of various Hospitals of Rawalpindi, after taking patient's written consent. Blood samples were collected through non probability convenient sampling. Samples were collected after approval from Ethical Committee of Army Medical College Rawalpindi. A consent Performa was signed by each patient before

sample collection. Study was conducted at center for Research in Experimental and Applied medicine (CREAM) in the Department of Biochemistry and Molecular Biology, Army Medical College.

Venous blood sample was collected from 15 co-infected patients of hepatitis Band C resistant to interferon therapy and from 15 healthy individuals. 5ml of blood was taken to perform RNA extraction and PCR. Written consent was taken from patients and controls and they were informed about the outcome of the study.

Total messenger RNA was isolated from blood sample using Ambion Pure Link RNA Mini Kit (Life technologies, USA). The first strand cDNA synthesis was carried out by using Revert Aid Premium First Strand cDNA Synthesis kit (Fermentas). The primers specific for STAT-1 were designed based on the previously available sequences on National Centre for Biotechnology Information (NCBI). The primer properties were analyzed using OligoCalc: Oligonucleotide Properties Calculator and Electronic PCR. The sequence of the primer is as follows: 6 F 5' GTCGGGGAATATTCAGAGCA 3' R 5' TGATCACTCTTTGCCACACC 3'[16]. PCR reaction mixture was prepared by adding 1µl of DNA/cDNA template. 2.5µl of 1x TBE buffer was added. 1.5mM of MgCl2 was added. 0.6mM of dNTPs were added. 0.3µl each of forward and reverse primer were added. 18.7 µl of nuclease free water was added. In the end 0.5µl of Taq polymerase was added. PCR was optimized through polymerase chain reaction technique on corbet Inc. PCR machine.

Data analysis was done by using SPSS version 20. Chi square test and descriptive analyses were performed to analyze the quantitative variables.

RESULTS

Total thirty subjects were included in our study. Fifteen of them were co-infected with hepatitis B and C as well as resistant to pegylated interferon therapy which was administered twice weekly for six months. Rest of the fifteen subjects were healthy individuals which were taken as controls. Among them 20 males that constitute 66.7% and 10 females that was 33.3% were included. Their ages ranged 21-54 and mean age was 35.90 ± 8.95.

The objective of this study was to detect STAT-1 in interferon resistant HBV/HCV co-infected patients. All the patients were diagnosed cases of chronic hepatitis B with super added hepatitis C infection or patients of chronic hepatitis C with superadded hepatitis B infection.

Total patients were 15. Among them 2 were female (13.3%) and 13 were male (86.7%). Their mean age was 39.46 ± 9.61. Total controls taken in the study were 15. Among them 8 were female (53.3%) and 7 were male (46.7%). Their mean age was 32.33 ± 6.79.

Comparison between patients and controls was done by chi square test andfisher exact probability value obtained was 0.500 which was not significant as shown in the table-1. PCR detection revealed that 67% of the patients expressed STAT-1 while 33% did not. Results of controls showed 57% detection of STAT-1 and 43% did not exhibit STAT-1 (fig-1).

DISCUSSION

Interferons are cytokines that regulate the expression of genes determining cellular fate and anti-viral defense. As the virus infects human body, interferons play a significant role in inhibiting viral replication but as infected cell produces insufficient interferon therefore it has to be given exogenously. Interferon binds with the receptor; Janus-Kinase phosphorylates tyrosine residues which then auto phosphorylates STATs proteins. They form homodimers or hetrodimers. These translocate into the nucleus and activate interferon response in the patient. This response varies in different individuals. Therefore interferon response should be studied extensively with special probe into the components of molecular signalling pathway. STATs are important in expression of genetic information against viral infection. As the results indicate increased expression of STAT-1; it could be due to multitude of factors involving cellular factors, viral and host factors. Different viral genotypes,

genetic diversity and viral load are viral factors which influence the response to interferon therapy.

In our study it has been detected that 67% of the patients were found to have STAT-1 in their blood, 33% of the patients did not express STAT-1 while STAT-1 was detected in 57% of the controls samples. These results are strengthened by the findings of a study conducted by Bin Gao et al. (2012) in France which revealed that STAT-1 levels are going to be higher as these are the markers of anti-viral response in human body. Another study complimenting the results of our study was performed in USA in 2002[17] which revealed an increase in STAT-1 detection in hepatitis B and C co-infected patients resistant to interferon therapy which indicate greater liver damage, fibrosis and an extensive and severer disease course in co-infection. In contrast, a study conducted in USA and Taiwan in 2007 revealed decreased STAT-1 in response to interferon gamma[18].

CONCLUSION

The results of our study showed that there was no significant difference in the presence of STAT-1 in HBV/HCV co-infected patients showing resistance to IFN therapy and in healthy individuals.

CONFLICT OF INTEREST

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

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