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ORIGINAL ARTICLE

Association of interferon-λ4-ss469415590 genotype with virologic response of interferon-alpha2b among chronic Hepatitis C patients attending a tertiary care hospital of D I Khan, KP, Pakistan

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ABSTRACT

Introduction: Hepatitis C virus (HCV) infection remains a hazard to universal public health. Treatment with interferon (IFN) plus ribavirin produces a sustained virologic response in about 50% patients after 6 months. Data regarding association of interferon with genotypes of virus and humans are still deficient.

Objective: To determine IFNL4-ss469415590 genotype in Hepatitis C patients and its association with virologic response to Interferon-alpha2b and Ribavirin treatment.

Materials & Methods: A cross sectional descriptive study was carried out from January 2012 to December 2014 in a tertiary care hospital of DI Khan, KP, on 165 chronic Hepatitis C patients enrolled through non-probability sampling after obtaining informed consent. Patients were given 300 IU of IFN-a-2a subcutaneously three times every week. Daily dose of ribavirin 1000 mg/day for a pre-treatment weight <75 kg or 1200 mg/day for weight >75 kg was also added. Relevant hematological, PCR and genotyping tests for HCV were done. Quantitative PCR tests were performed before treatment and at 1 month, 3 months and 6 months post-treatment for each patient. Taqman assay-based SNP genotyping was done for detection of the allelic variants at rs368234815. Real time PCR was used to amplify patients' DNA and SNP analysis for single nucleotide mutation using PCR thermal cycler SDS7000.

Results: At rs368234815, 85(51.5%) were Responders. Genotype 3a(51%) and 2b(20%) were the predominant genotypes. Among responders, 43(50.6%) patients were having TT genotype, 28(33%) GT and 07(8.2%) GG genotype. Among 80(48.5%) non-responders, 15(18.8%) had TT genotype, 52(65%) GT and 11(13.7%) GG genotype. TT genotype presence displayed a significant association (p=0.003) with treatment result i.e. TT > GT >GG.

Conclusion: The TT genotype at SNP rs368234815 offers improved virologic response to interferon in combination with ribavirin in chronic Hepatitis C patients when compared to other genotypes.

Keywords: Interferon-λ4-ss469415590; HCV; Lambda 4 (λ4); Genotype; Interferon; Ribavirin.

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Hepatitis is defined as a condition characterized by inflammation of the liver, usually due to viral infection. Various kinds of hepatitis are classified according to etiology; mostly Hepatitis A, Hepatitis B and Hepatitis C viruses being the main causes of the disease.[1]

In 2011, WHO report revealed that Hepatitis C virus infected about 170 million people worldwide, making HCV as the main reason leading to chronic liver disease. Most acute infections persists unnoticed due to absence of symptoms making it difficult to calculate the prevalence of infection. In many countries there is deficiency of satisfactory community-based disease reporting systems and selected groups are chosen to perform studies; this results in incomplete details of the overall prevalence, including drug users and blood donors.[2,3] In Pakistan, more than 10 million people are affected by the disease;[4] **t**he most prevalent HCV genotype in Pakistani population is 3a[5] followed by other genotypes.[6]

Our immunity plays a vital role in wiping out 15-20% of acute HCV infections spontaneously, but once disease becomes chronic there is little chance of its spontaneous abolition. No vaccine is available for HCV till date. The treatment options available for chronic HCV are combination treatments with Pegylated Interferon Alpha (IFN-α) & Ribavirin and Sofosbuvir. The treatment response is influenced by numerous elements; these include Gender, Age, Hepatitis Virus Genotype, Viral Load & Liver Fibrosis. In case liver cirrhosis has occurred, liver transplantation is the only choice.

IL28 locus of human interferon gene has numerous polymorphisms which can encode a kind of IFN-λ associated with treatment results.[7] Ribavirin is only effective when used in combination with IFN-α.[8]

Another proposed mechanism of antiviral effect of Ribavirin is that it acts as an RNA mutagen, resulting in viral mutations that cause virus destruction through mechanisms named as "error catastrophe".[9]

It is assumed that "Single nucleotide polymorphism of IFNL4-ss469415590 is a strong predictor of response to IFN-RBV regimen in chronic Hepatitis C patients."

The present study was designed to determine IFNL4-ss469415590 patient genotype and to find possible association of IFNL4-ss469415590 genotype with virologic response to interferon-alpha2b and ribavirin treatment.

MATERIALS & METHODS

A three-years cross sectional descriptive study was carried out from January 2012 to December 2014 on 165 Hepatitis C positive patients in a tertiary care hospital of Dera Ismail Khan (DI Khan), Khyber Pakhtunkhwa (KP), in collaboration with the Institute of Basic Medical Science (IBMS), Khyber Medical University (KMU), Peshawar. All seropositive patients for anti-HCV by third generation ELISA (Enzyme Linked Immunosorbent Assay) were included in the study while patients above 50 years, with BMI less than 18.5 & above 30, patients with liver cirrhosis, alcoholic patients and those patients having co-infections such as HBV/HIV were excluded. Patient enrollment was done through non-probability sampling after taking written consent. Full blood counts were done to evaluate and measure several components that included hemoglobin, Total Leukocyte Count, Red Blood Cells, neutrophils, lymphocytes, monocytes, and platelets, MCV, PCV, MCH. PCR; genotyping tests for HCV were done. All patients received 300 IU-IFN-a-2a subcutaneously three times a week. Daily dose of ribavirin (1000 mg/day for a pre-treatment weight <75 kg or1200 mg/day for weight >75 kg) was also given. Quantitative PCR tests were performed for each patient before treatment and after 1 month, 3 months and 6 months of treatment. For the detection of the allelic variant at rs368234815, Taqman assay-based SNP genotyping protocol was adopted. Real time PCR was used to amplify patients' DNA and SNP analysis done for single nucleotide mutation. PCR was achieved on Real time PCR Thermal Cycler SDS7000. After termination of therapy patients were monitored weekly for 6 months.

Statistical Analysis

Graph pad Prism and Microsoft Excel 2010 were used for descriptive statistics of genotypes and alleles. Fischer Exact test and Chi-square tests were performed to find association of alleles. Odds ratio and 95% confidence interval for genotypes were calculated to determine size and effects of alleles and genotypes. Software programs MedCalc and Contingency Table were used for this purpose.

RESULTS

Of the 165 HCV infected patients undergoing interferon treatment included in the study, 89(54%) were male and 76(46%) were female. A high incidence (40.6%) of HCV infection between the age group of 41-50 years was noted (Figure 1).

Different countries show different Hepatitis C virus genotype distribution. In Pakistan the dominant genotype is 3a. After genotyping, the samples had predominantly 3a(45%) and 3b(19%) genotype, followed by 2a(13%), 1a(6%), 2b(8%), 4a(1%), while 5a and 6a were 0.5%. Some of the untypable (10%) were also present. Distribution of viral genotype among responders and non-responders is shown in Figure 2 and Figure 3.

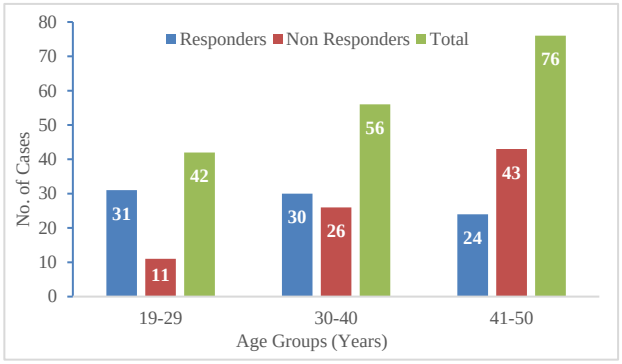

Figure 1: Relationship between age groups and response

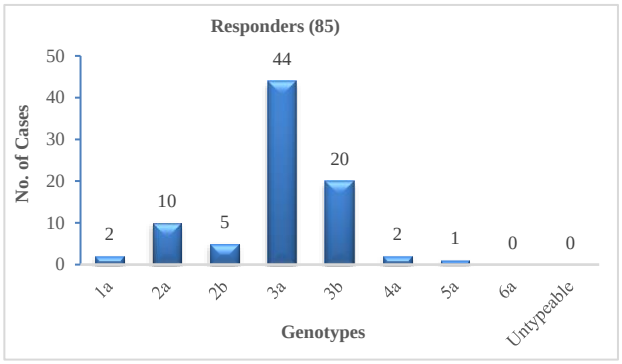

Figure 2: Viral Genotypic Distribution among Responders

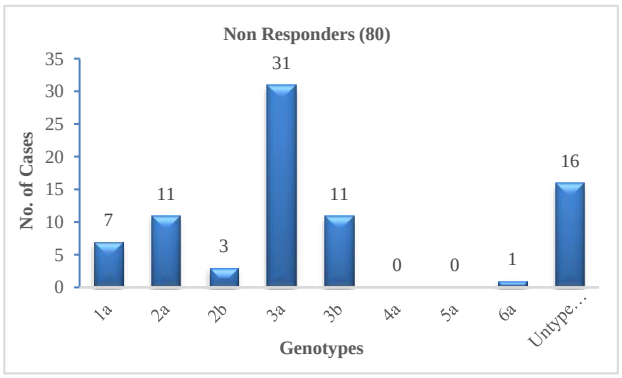

Figure 3: Viral Genotypic Distribution among Non-Responder

Considering the different category of viral load such as Low level (< 600,000 IU/ml), Intermediate level (600,000 - 800,000 IU/ml) and high level (>80,0000 IU/ml), it was found that viral load before treatment for non-responders was 11106.01 - 8921478 IU/ml and for responders it was 36921 - 6584129 IU/ml. After six month of Interferon treatment the range of viral load was 18000 - 75896345 IU/ml for non-responders and 0 for responders as shown in (Table 1).

Table 1: Viral load of responders and non-responders before and after treatment

Viral load	Before treatment (IU/ml)	After 6 months treatment (IU/ml)
Non-Responders	11106.01 - 8921478	18000 - 75896345
Responders	36921 - 6584129	0

At rs368234815 Taqman Assay, out of total 165 patients 85 were responders. The allelic determination through Taqman assay

provided graphic results for non-responders (Figure 4**)** and for responders (Figure 5).

In allelic discrimination graph of non-responders (figure 4) blue color represents allele G while green represents both alleles GT.

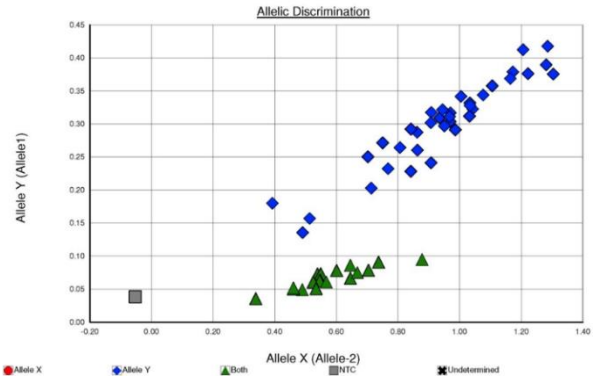

Figure 4: Allelic discrimination assay for non-responders

While in the graph of responders (Figure 5) blue color represents allele G, green color both GT while red color represents allele T.

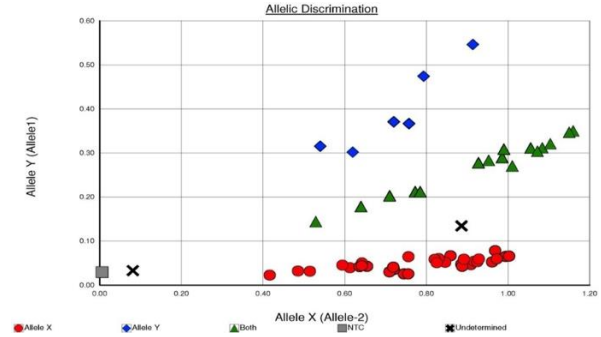

Figure 5: Allelic discrimination assay responders

Among responders 7(8.2%) samples were untypable, 43(50.6%) patients were having TT genotype, 28(33%) had GT and 7(8.2%) had GG genotype. Non Responders were 80, out of which 1(2.5%) was untypable, 15(18.8%) patients were having TT genotype, 52(65%) GT and 11(13.7%) were having genotype GG. Study provided a strong associated of TT/TT genotype with responders and G/TT with non-responder with a significant difference of genotypes P value p=0.438578, p=0.002931 for responders and non-responders respectively (Table 2).

Table 2: Results of genotyping for rs368234815

Genotypes	Responders	Non Responders	p value
TT/TT	43	15	<0.0001
G/TT	28	52	
GG	07	11	
Total	78	78	
p-value	*p*=0.438578	*p*=0.002931	

The homozygous genotype TT/TT (114 alleles 58%) are better responders as compared to heterozygous genotype TT/G (24 alleles 36.2%).The homozygous genotype TT/TT responds a lot more to interferon as compared to heterozygous TT/G and GG. The GG against TT Odds ratio were 2.449 on Fisher's exact test for association of alleles with drug response showing the significant difference p=0.003 between responders and non-responders (Table 3). Association of genotypes was checked by Chi-square test, p=0.0001 was found (Table 4).

Table 3: Showing rs815 genotype GG against TT

Alleles	Responders	Non Responders	Association	Test variables		
				p value	95% CI	Odds ratio
TT	114	82	ΔG vs TT	0.0003	1.526-3.9	2.449
ΔG	42	74				

Table 4: Chi-square test done for association of genotypes

Genotypes	Responders	Non-responders	Association	Test variables	
				p value	df
ΔG	42	74	ΔG VS TT	0.0001	21.61, 2
TT	114	82			

DISCUSSION

The study was conducted on chronic HCV patients to determine the association of Interleukin-λ4-ss469415590 genotype with virologic response to Interferon-alpha 2b. The Pegylated Interferon and Ribavirin treatment responses were very much effective at the start after its commencement in 1998[10,11] but with the passage of time the concern regarding sustained virologic response (SVR) of pegylated interferon therapy increased.[12-14]

Present study explored 3a and 2b HCV genotypes infection predominant among infected patients of DI Khan region with 50% SVR rate**.** Other studies conducted in Pakistan justified the finding of this study describing the 3a and 2b a predominant HCV genotype among Pakistani infected population.[15]

Present study showed that in older age (>40 years) the non-responders frequency were higher than responders**.** Similarly, another study reported that with increasing age the non-response rate of HCV treatment increases.[16] A study conducted in Pakistan showed that SVR rate were high among older age HCV patient compared to lower age patient.[17]

Both treatment responders and non-responders groups of chronic HCV patients present high viral loads (> 800,000 IU/ml) before

treatment thereby negating the importance of high viral load on treatment response. In the present study viral loads ranged from low to high levels before treatment.

The present study also found that rs368234815, genotype TT/TT was a strong predictor of SVR in patients suffering from HCV; thus there are more chances of SVR, TT>GT>GG (Table 3). In the past few years different studies worldwide have shown almost similar results. The results carried out in different countries on different ethnic groups support the current research work.[18,19] The present study has also supported the finding that TT genotype presence indicates a valid association with treatment results i.e. TT > GT >GG at rs815.

The present study is consistent with other studies in exploration of TT/TT genotype a dominant allele among responders and TT/G allele in non-responder of Pakistani population.

CONCLUSION

The TT genotype at SNP rs368234815 offers improved sustained virologic response (SVR) in chronic Hepatitis C patients who are on Interferon & Ribavirin combination therapy. In HCV patients of DI Khan the non-response rate to interferon treatment was high and a significant association of non-response with TT/G alleles was observed.

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