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Association of IL28B rs12979860 and rs8099917 Gene Polymorphisms with Response to Combination Therapy of Sofosbuvir and Daclatasvir in HCV-Infected Patients from the Khyber Pakhtunkhwa Population

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Abstract:
Objective: The aim of the study is to analyze if there is are any polymorphisms in IL28B rs12979860 and rs8099917.
Methodology: This is a descriptive study of an emerging phenomenon. Therefore all patients who are diagnosed with chronic Hepatitis C and found resistant to sofosbuvir and Daclatasvir by PCR after at least 12 weeks of initiating treatment and fulfilling our inclusion criteria were recruited in group 1. All those patients who responded to therapy and had negative PCR at 12 weeks were recruited in group 2. Peripheral blood was taken from the patients and kept in ETDA plastic tubes for analysis of IL28B genomic DNA extraction by PCR. The PCR products were then sequenced and analyzed.
Results: No polymorphism was seen in rs12979860. Polymorphism was seen. In rs8099917 however it was not significant
Conclusion: IL28B significantly impacts the immune response to HCV and modulates the interaction of genetic variations with host and viral factors, ultimately influencing treatment outcomes.

Introduction:
An estimated 71 million people worldwide are living with chronic HCV infection, representing a major public health and economic burden(1). A small subset of patients achieve spontaneous viral clearance whereas 60–80% develop chronic infection with 20–40% progressing to cirrhosis or hepatocellular carcinoma(2-4).

HCV is 9.6kb ssRNA enveloped virus and belongs to family Flaviviridae. It is because of HCV RNA dependant RNA polymerase (NS5B protein) the virus exhibits a high level of sequence heterogenicity(5). On basis of sequence homology it has been identified into 6 major genotypes and numerous subtypes. Distribution of genotypes varies geographically. There is a global distribution of genotype 1 and 3, whereas genotype 2, 4 and 5 are common in Middle East and Africa while genotype 6 prevails predominantly in South East Asian countries(5-7)

Pakistan has third highest burden of HCV in the world with adults affected by HCV in 4.8% of population which accounts for around 8–10 million people. This high prevalence ranks Pakistan among the most HCV-endemic countries worldwide, making HCV a significant public health issue(8, 9).

Before the advent of DAA pegylated interferon was used with or without interferon achieving 40-45% of SVR. However, patients used to have severe side effects such as depression, anemia and weight loss. After the development and approval of direct-acting antivirals (DAAs) in 2011 by FDA and its ability to directly targeting the viral sites including NS5A inhibitors, NS5B polymerase inhibitors, DAA became the drugs of choice for treatment choice in hepatitis C patients(10, 11).
The outcome of HCV treatment also depends on Host factors which included that stage of the disease process, comorbidities, coinfections and genetic variations in IL28B. Studies have shown that a single nucleotide polymorphism (SNP) in the interleukin-28B (IL28B) gene strongly correlates with spontaneous viral clearance and SVR in patients treated with PEG-IFN-α and RBV(12).
The target goal of the present time study is to evaluate if there is any role of IL28B rs12979860 and rs8099917 genetic polymorphisms as potential markers for predicting DAA treatment response in HCV patients.

Objective:
To find out polymorphisms occurring in IL28B gene rs12979860 and r8099917 in patients responding and resisting to Sofosbuvir and daclatasvir.

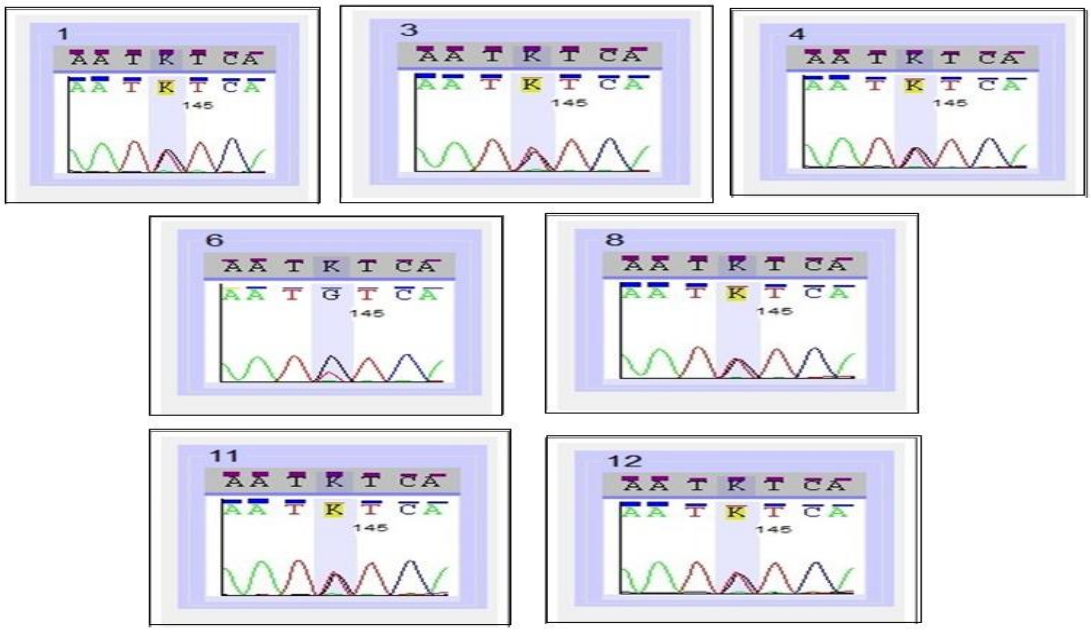
Material and method
After obtaining approval from the ASRB, informed written consent was collected from all participants before enrollment in the study. The patients were categorized into two groups, each comprising six individuals:

- **Group 1**: Patients who responded to SOF and DCV after 12 weeks of treatment.
- **Group 2**: Patients who developed resistance to SOF and DCV after 12 weeks of treatment.

To analyze SNPs in IL28B, 3–5 mL of blood was collected in plastic EDTA vials from both responder and non-responder groups. Samples were stored at -80°C until genomic DNA was extracted.Primer design was carried out using the UCSC Genome Browser to determine the melting temperature (Tm) and specificity for each binding primer pair. DNA was isolated using the standard organic phenol-chloroform method. The quality and quantity of the isolated DNA were assessed prior to performing agarose gel electrophoresis and PCR, which was conducted using Taq Green Master Mix (Thermo Fisher Scientific Inc., USA).PCR products were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc., USA). Sequenced data were analyzed using Chromas Lite software (v2.0). Any variations in sequencing results were cross-verified using Sequence Server and the BLAST (Basic Local Alignment Search Tool) feature on the NCBI database to identify SNPs in IL28B, specifically rs12979860 and rs8099917

Results
Polymorphism rs8099917 c.252 T > G was found in the heterozygous state in 7 patients while the remaining 5 patients showed the wild type of rs8099917.

Figure :1,3,4,6,8,11, and 12 were found with rs8099917 (c.252 T > G). The figure representsthe electropherograms of the patients showing heterozygous state of rs8099917



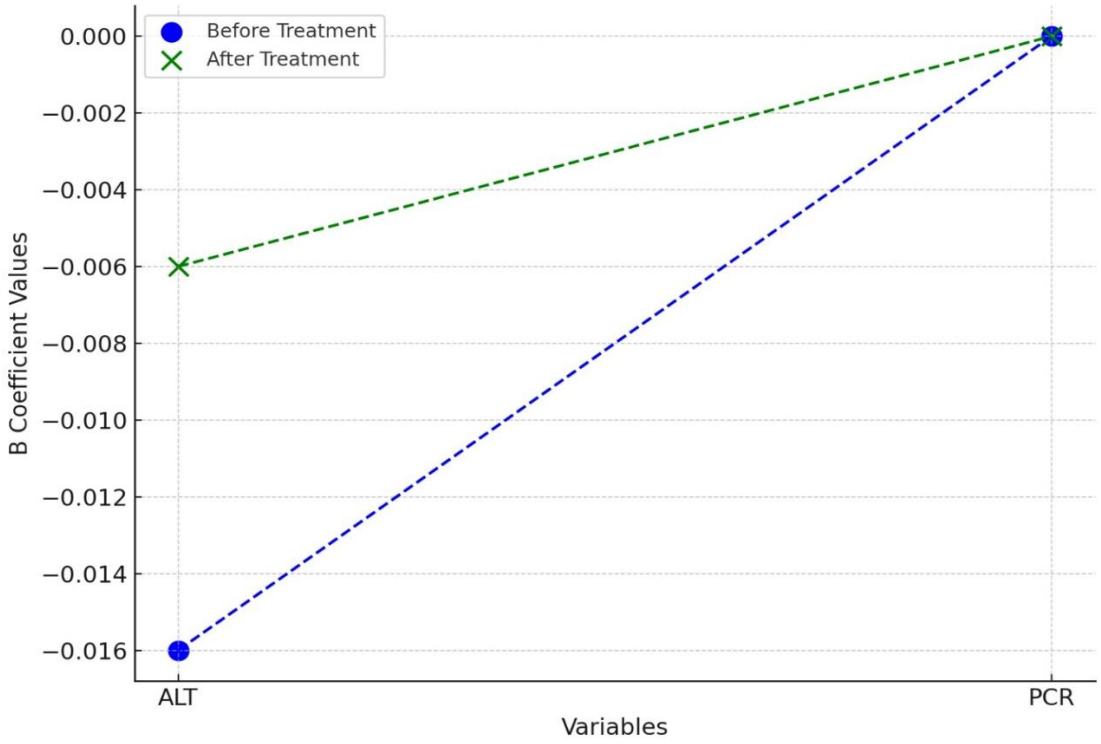
In our patients no rs12979860 polymorphism was found. In order to confirm, the sequenced data was aligned with the reference sequence. The same process was repeated and the targeted sequence was downloaded from UCSC genome browser. Human genome GRC Ch38 assembly was used as reference. We used a comprehensive bioinformatics software platform Geneious® 9.1.8 designed for sequence analysis. The alignment process was commenced by importing the high-quality trimmed sequences into Geneious and the reference genome sequence was also loaded. The integrated **MUSCLE** (Multiple Sequence Comparison by Log-Expectation) algorithm was used for multiple sequence alignment. The alignment results were visually inspected within Geneious to identify any discrepancies or areas of interest, such as regions of high variability or conservation. After the alignment, we found that there is no SNP detected. The figure 3.10 represents the alignment of the sequences for rs12979860.

Multiple sequence alignment for rs12979860 polymorphism in IL28B gene.

Before Treatement								
Parameter	**Coefficient**	**St. error**	***X*² statistic**	**df**	***p*-value**	**Odds ratio**	**95% C.I.for EXP(B)**	
							Lower	**Upper**
Baseline ALT	-.016	.011	2.393	1	.122	.984	.963	1.004
Baseline PCR	.000	.000	2.197	1	.138	1.000	1.000	1.000
Constant	2.862	2.478	1.334	1	.248	17.496		
After treatment								
Parameter	**Coefficient**	**St.error**	***X*² statistic**	**df**	**p-value**	**Odds ratio**	**95% C.I.for EXP(B)**	

							Lower	Upper
Week 12 Alt	-.006	404.207	.000	1	1.000	.994	.000	.
Week 12 PCR	.000	.012	.000	1	.995	1.000	.976	1.024
Constant	49.273	30633.601	.000	1	.999	25058761938682847000000.000		

Association of ALT and PCR of rs8099917.



Discussion

Hepatitis C infection is of great concern throughout the world bearing an immense cost over national and international level. The infection acts like a silent killer, by destroying the liver rapidly while being symptom free till it advances. The severity of infections depends upon several factors that includes viral and host factors, IL28B being one(13). IL28B is known to have some protective role as it has some antiviral activity but SNPs related to it leads to failure in treatment with certain drugs like sofosbuvir and daclatasvir(14).

The exact mechanism of association between IL28B and DAAs is still unknow but it is suggested that the antiviral drugs might initiate the JAK STAT pathway which has some antiviral properties. Those patients who might carry an allele that's unfavorable allele of IL28B might not respond towards the direct antiviral agents(15).

In our study we did not detect any polymorphism in rs12979860. Our study results our consistent with another study from Egypt who also concluded that there was no polymorphism detected and hence it does not affect the treatment response with SOF+DCV(16).

Some polymorphism was detected in rs8099917 however it was not statistically significant, interestingly another SNP rs8113007 was detected and it showed polymorphism in patients who were undergoing sofosbuvir and daclatasvir treatment. SNP's rs12979860 and rs8099917 are the most extensively studied to evaluate the occurrence of chronic HCV infection. In this study conducted on Pakistani population we found the rs8099917 (c.252 T > G) and another SNP rs8113007 (c.190 A > T). Whereas no detection of rs12979860 was found in the Pakistani population.

Conclusion

Research is required to fully elucidate the mechanisms through which IL28B influences the immune response to HCV, as well as to explore how these genetic variations interact with other host and viral factors in determining treatment success. Understanding these genetic determinants will continue to be important for optimizing personalized treatment approaches and improving the outcomes of HCV therapy worldwide.

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