Review Article

Clinical Immunology & Research

Gene Polymorphism of Responder, Relapser and Non-Responder of Chronic Hcv Infection among Ethnic Tribes of Punjab, Pakistan

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ABSTRACT

Objectives: *To study the susceptible gene in reference to the treatment of relapsers & non-responders various ethnic tribes. To determines the role of IL-10 and IL-28B gene polymorphism in ethnic group of Punjab.*

Subjects & Methods: *Total of 402 patients of HCV was recruited for this study. Patients diagnosed anti-HCV positive confirmed by HCV PCR qualitative test. All these selected patients diagnosed for HCV PCR (qualitative & quantitative), genotyping. Who have taken the standard treatment of IFN/Ribavirin 20-36 weeks. Objective of the study was to find the role of gene susceptible in responders, relapsers & non-responders of HCV. Study was undertaken to find the role of gene Polymorphism of IL10 and IL28B BstU 1, Rsa and Bst61 enzymes were used to digest the PCR amplicons.*

Results: *There is an association of cytokine gene polymorphism and susceptibility to hepatitis C infection. The gene polymorphism of different genetic variants as IL10 and IL28B were the most important genetic variants. A clear difference of polymorphism found in both groups, i.e., GA polymorphism in 132 of relapser & 68 in non-responder. AA was 22 in relapser & 14 in non-responder. This is clear that gene polymorphism was more frequent in relapser than in non-responder. These two genotypes i.e., IL10 and IL28B played important role in the eradication of HCV. Therefore, treatment needs 48 weeks rather than 24 weeks' time, which is a normal duration of treatment.*

Conclusion: *This research found that gene polymorphism impart as a major contributing factor to do testing of these IL10&IL28B positions to achieve the target therapy of non-responders and relapsers (Rubi & Aslamkhan, 2016).*

Introduction

HCV is a blood borne RNA viral disease, primarily spread by direct contact with blood or body fluids of infected individuals [1]. Acute HCV infection is difficult to detect because it is generally asymptomatic nature &the marginalization of at-risk population (silent killer). Major viral hepatitis as HBV & HCV are major epidemics of Pakistan. As 15 million people have been infected with HBV /HCV. All of us aware of major cause of this infection is misuse of needles, syringes, unscreened blood transfusion, sharing of contaminated items as brush, cutters, shaving items, pre & post op procedures, dental treatment, parlor & massage items or etc. In addition, massive use of injections [2,3]. It is estimated that 3.3% of the world's population HCV infected, 7.5% in Pakistan. As 195 million infected with HCV as a global health problem [4,5]. Due to high prevalence of HCV chronic infections, there is a need to understand its epidemiology, more people die annually with Hepatitis C than HIV, the virus that causes AIDS; but Hepatitis C education pales in comparison. It warns world for prevention & control of spread. HCV prevalence is highest in Egypt at >10% of population & China has HCV infection 9%. Countries with high incidence of HCV or peak incidence further escalations in HCV-related cirrhosis & HCC. 25% of patients with acute HCV infection undergo spontaneous clearance. While 75% of patients' progress to chronic HCV infection at risk to hepatic fibrosis, cirrhosis & HCC [6,7]. Main cause of liver transplant is HCV infection.

HCV Genome

This genome belongs to the Flaviviridae family; it has some similar structural & virological characteristics of this family. It is covered in a double layer of lipids with two or more proteins enclosed in it [5-7]. This envelops enclose nucleo capsid made up of many copies of basic protein core & then it enclose RNA genome. The flaviviridae genome is a positive strand of RNA molecule. Size ranges [9]. 6-12000 nucleotides with ORF. These structural proteins covered with NS5 region, while the rest of the part code for non-structural proteins. Similarities are of Flaviviridae genera, but HCV also shows some differences as virological, epidemiological as well as patho physiological [8,9].

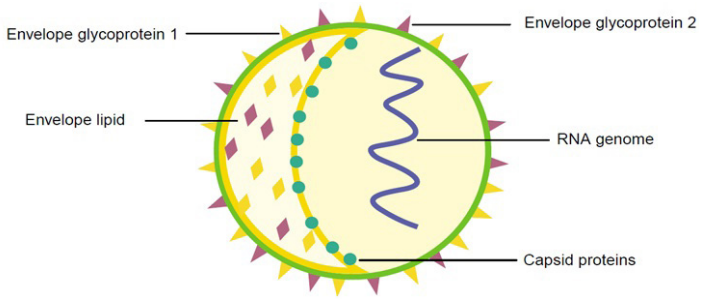

Figure 1: Showing the structure of HCV Genome [10].

Genotype of HCV	Distribution of genotype worldwide
1a	First genotype is found in North and South America, Australia, and in South Asia
1b	It is mostly found in Europe and Asia
2a	This genotype is found in Japan and China
2b	This 2b genotype of HCV is found in US and North Europe
2c	This Genotype is found in Western and Southern Europe
3a	Highly prevalent Genotype found in Australia (40% of cases) and South Asia, including Pakistan
4a	The most prevalent genotype of Egypt
4c	Most common genotype found in Central Africa
5a	The high prevalent genotype found only in South Africa
6a	This genotype is restricted only to Hong Kong, Macau and Vietnam
7a and 7b	Genotype which is Common in Thailand
8a, 8b and 9a	These three subtypes of Genotypes are Prevalent in Vietnam

Table 1: Shows the worldwide HCV Genotype Distribution [11].

Rational

Genetic Susceptibility of HCV RNA gene is critical according to the host of patients of HCV as it depends on genetic variation to predict the kind of interferon treatment, Response to treatment and spontaneous clearance of viral load. Interleukin gene polymorphism has respective contribution of direct HCV treatment by interferon. Various studies done in contest to IL10 and IL28b genes. These studies aid in interferon treatment/mixed therapy to clear the virus, rather than creating non-responders and relapser in this society.

Subjects & Methods

Total of 402 patients of HCV were recruited for this study, but 53 refused to proceed with this study. Patients diagnosed anti-HCV positive confirmed by HCV PCR qualitative test. All these selected Patients diagnosed for HCV. Who have taken the standard treatment of IFN/Ribavirin 20 weeks - 36 weeks? After the formation of three groups of patients. Both males and females, 18-56 years old, belonging to diff districts of Punjab, selected for study. The patients with mixed infection of HBV/HIV or receiving Interferon treatment, not included in the study. (Exclusion Criteria). Only patients diagnosed having HCV infection, who received the treatment with standard alpha interferon, were studied. The patients, who after the treatment of interferon therapy respond successfully recovered. HCV PCR test result became negative labeled as "cured", called as Responder. Whereas the patients, who got the standard therapy of interferon, responded/ recovered very well but turned up in HCV clinics after 6-24 months showing the symptoms of HCV known as "recurrence" of virus. Their PCR test also indicated positive were relapser cases. Whereas the third group of patients did not respond to the therapy even after 16 to 24 weeks of treatment, called as non-responders.

Consent Form

The data of each patient recorded on a questionnaire. DNA extraction and molecular analysis performed in the Laboratory. Patients were informed about the research and procedure of sample collection, and then they were requested to participate voluntarily in this research project. Those who agreed after reading the consent form (in English/Urdu) or listening to its contents were asked to sign it. The data of each patient was recorded on a questionnaire. The complete clinical history of all these were taken. Laboratory investigation data of liver enzymes, ELISA report of anti-HCV & confirmatory test of HCV PCR done. DNA extraction and molecular analysis were performed in Human Genetics & Molecular Biology Department at The University of Health Sciences, Lahore.

Blood Samples

Using aseptic measures, 5 ml peripheral venous blood samples taken from diagnosed patients of chronic HCV infection. Responders, relapser and non-responder patients. Samples were collected in standard EDTA vacutainers and serum collection tubes, (DNase free tubes).

DNA Extraction

Genomic DNA was extracted from all collected blood samples by using the salting out method, described by [12]. The extracted DNA stored at -20oC until further used. Qualitative & Quantification of DNA done by using "Agarose DNA Gel Electrophoresis method". Quality & Quantity of DNA extracted estimated by 2% agarose gel. This study focused only on HCV patients, who were non-responsive and had relapsed from conventional therapy of standard interferon treatment. Objective of the study was to find gene Polymorphism in patients of HCV, Study was undertaken to find the role of gene Polymorphism of IL10 and IL28B. The gene polymorphism of different genetic variants as IL10 and IL28B were the most important genetic variants. As there is an association of cytokine gene polymorphism and susceptibility to

hepatitis C infection.

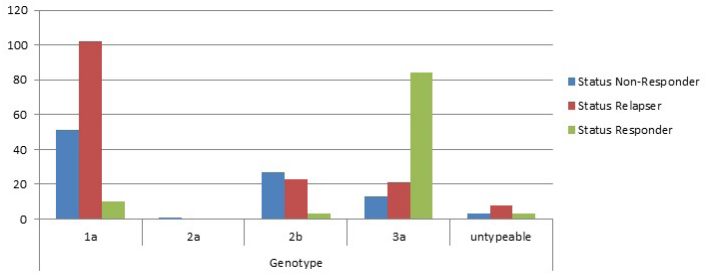

Figure 2: Showing the status of Patients with HCV Genotypes Distribution.

HCV RNA Gene Susceptibility plays a vital role for Patients HCV. As it depends on the variations of gene. It also determines the type of therapy/kind of interferon to clear virus. The ultimate goal for all the studies of HCV is to uncover how this virus germinates & to eradicate the HCV infection. Also to inhibit the replication of HCV RNA viremia. To focus on the study of the genes of IL-10 & IL-28B. Therefore, Polymorphism of those genes is very important. SNP in the region of IL-10 on chromosome 1 & IL-28B on the chromosome 19, as it codes for the IFN alpha. IL-28B is strongly associated with treatment of interferon& ribavirin for patients of HCV with genotype 1 & 3. There is an association of cytokine gene polymorphism and susceptibility to hepatitis C infection [1,13].

RFLP of IL-10 (rs 1800871)

To amplify the region of IL-10 gene as IL10-1082, IL10-819, IL10-592, So, Restriction digestion were done to detect three SNPs.

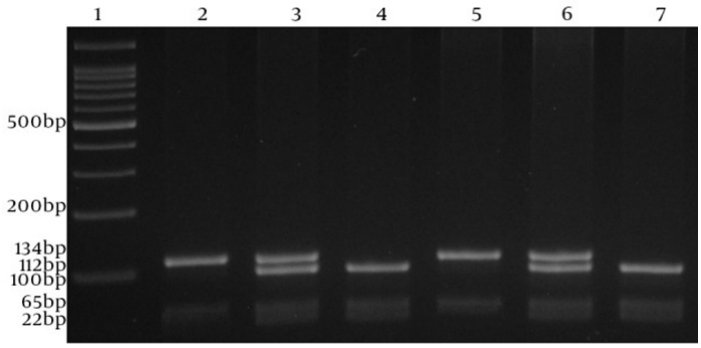

Figure 3: Shows SNP of IL10-1082.

Restriction Digestion at 1082 G/A, as Enzyme Ear I cut 112bp ⟶ 90bp & 22bp.

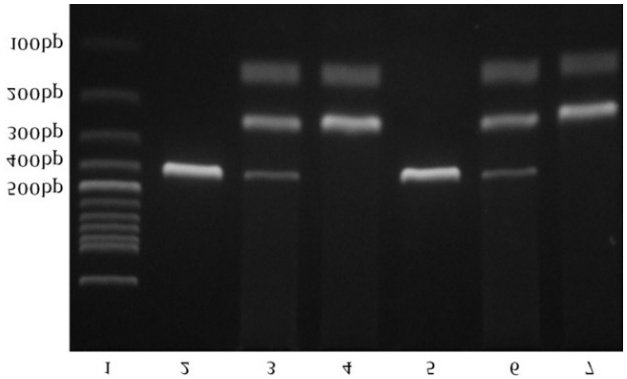

Figure 3: shows SNPs of IL10-819.

Restriction Digestion at 819 A/C by enzyme Mae III cut 300bp ⟶ 170bp &130bp.

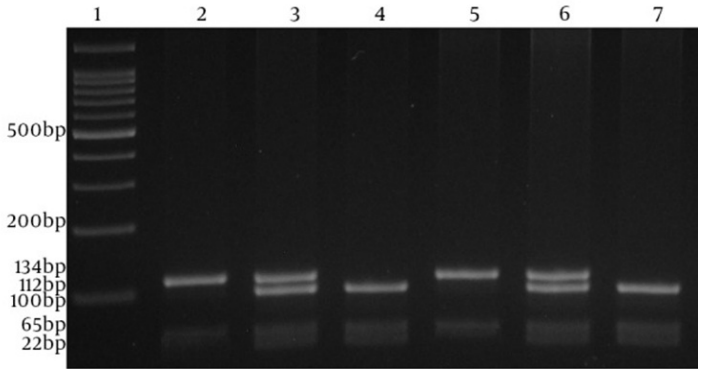

Figure 5: Show SNPs of IL10-592.

Restriction Digestion at 592 C/T by enzyme RsaI cut 112bp ⟶ 78bp & 34bp.

Gene	SNPs	Polymorphism	Frequency	Percentage
		AA	36	14.5
	IL10-1082	GA	200	80.3
	rs1800896	GG	13	5.2
		AA	8	3.2
IL-10	IL10-819	AC	211	84.7
	rs 1800871	CC	30	12.0
		AA	15	6.0
	IL10-592	CA	174	69.9
	rs 1800872	CC	60	24.1

Table 2: showing the Frequencies of IL-10 SNP's Polymorphism.

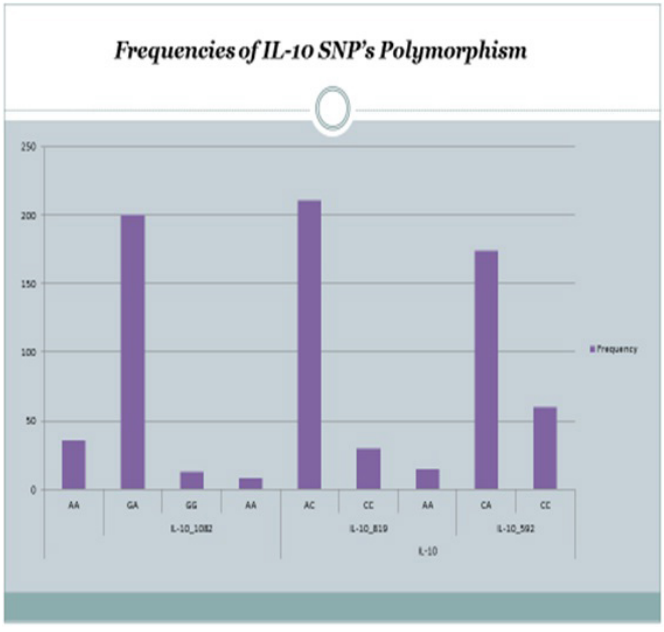

Figure 6: Shows the Polymorphism of IL-28B.

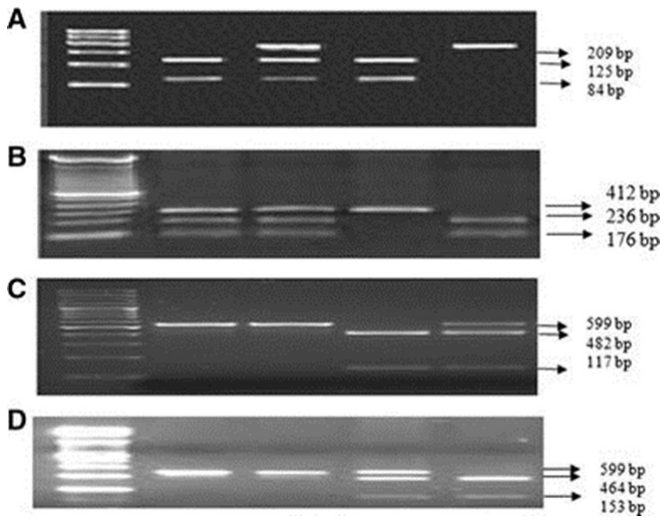

Figure 7: Shows the Polymorphism of IL-28B.

Image A shows BstU1 cut 209bp into 125bp &84bp fragments. Image B shows digestion of Rsa 412bp into 236bp &176bp fragments. Image C shows Bst 61 cut 599bp into 482bp &117bp fragments, while another cut in image D seen 599bp into 464 & 153bp.

Gene	SNPs	Polymorphism	Frequency
GG	12	4.8	
IL28B a	TG	101	40.6
(rs 12979860)	TT	136	54.6
	CC	87	34.9
IL28B b	CT	145	58.2
(rs 8099917)	TT	17	6.8
	CC	100	40.2
IL28B c	CT	109	43.8
(rs 12980275)	TT	40	16.1

Table 3: Showing the Frequencies of IL-28B SNP's Polymorphism.

Human genetic susceptibility to HCV genotypes appears to be of important in getting the infection. The study suggests i.e. Gene Polymorphism of IL-10 & IL-28B interleukin genes should be tested prior to the treatment.

Discussion

Human genome contains more than 3 billion nucleotides with over 10 million single nucleotide polymorphisms (SNPs) [5]. Genetic polymorphism assessed by RFLP which covers more than 90% of common genetic variation [14]. The ability to test millions of SNPs per individual leads to the challenge of multiple testing. Therefore, a well characterized clinical cohort with clearly defined phenotypes are critical to avoid spurious results and genetic associations [15]. Though HCV is not the genetic disease but susceptibility of disease is genetics [3]. The study was carried out to find the gene which was most susceptible in the population of the Punjab. The susceptibility of HCV is totally dependent on the genotype of HCV [16], and the polymorphism of the gene mostly affects the susceptibility to chronic infection of HCV as suggested by some scientists [17,18].

A clear difference of polymorphism found in both groups, i.e., GA polymorphism in 132 of relapser & 68 in non-responder. AA was 22 in relapser & 14 in non-responder. Hence, this is clear that gene polymorphism was more frequent in relapser than in non-responder. These two genotypes i.e., IL10 and IL28B played a very important role in the eradication of Hepatitis C virus. Therefore, treatment needs 48 week rather than 24 week time, and that is the normal duration of treatment.

Conclusion

We determined that HCV Genotype 3a is precisely communal as (84.0 %) amongst group of responders, whereas Genotype 1a is more common in relapser (66.0 %), While non-responders (54.0 %). Genotype 4 was excluded, the remaining 5 major genotypes, known as, 1a (61.40 %), 2a (0.50 %), 2b (20.00 %), 3a (13.70 %) & unidentified (4.40 %) were amongst the twelve various groups of Pakistan, as reported by Zhao Y, et al. and Chung-F Huang and Chia-Y Dai [18,19].

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