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Role of Interleukin 10 in Hepatitis C Viral Clearance and Distribution of its Polymorphism in Pakistani Population

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ABSTRACT: Hepatitis is a chronic viral disease. HCV is the most escalating disease in Pakistan. It is caused by poor medicating strategies; unsterilized operating utensils, syringes etc. Its genome consists of RNA dependent RNA polymerase that does not contain any proofreading activity. Thus there are various strains of HCV. HCV infection is described by sustained or rapid response of viral particle. Severity of HCV infection directly linked with genome. Recent studies show that there are some cytokines producing genes that involve in antiviral therapy. One the most important cytokine is IL-10 (interleukin 10). 1082G/A, 819C/T and 592A/C polymorphisms found at promoter region of IL-10 gene. Different polymorphism (1082AA, 592CC and 819CC) produces high level of IL-10. So, they facilitate our immune system to reduce severity of HCV infection among patients. Furthermore, different treatment strategies of HCV infection will also be discussed in this review article. There are multiple causes of HCV occurrence in people. It is very difficult to pretreat HCV susceptibility. But now a days evolving science of genetics can solve this problem. Genome analysis can tell us about susceptibility of a person to HCV infection on base of polymorphism at genes responsible against viral defence system.

Keywords: Polymorphism, cytokines, sustained viral response (SVR), rapid viral response (RVR), hepatomegaly, splenomegaly, RNA dependent RNA polymerase (RdRp), lysogenic cycle, lytic cycle, pro-inflammatory cytokines, vitamin D receptors (VDR), T Helper Cell Type 1 (TH1), T Helper Cell Type 2 (TH2).

I. INTRODUCTION

Hepatitis is such a chronic complication which leads to different hepatic problems. Hepatitis is a viral disease that is transmitted through blood, plasma, urine and even saliva [1]. There are various types of hepatitis depends on the order of discovery. Different types of hepatitis are Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis E, Hepatitis F, Hepatitis G, Hepatitis H, autoimmune hepatitis and alcoholic hepatitis. Most prevailing type of hepatitis in Pakistan is HCV and HBV infection [2]. There are a great list of medicines to treat HCV with least chances of recovery. These medicines also have side effects. In this article we are trying to interrelate severity of HCV and gene polymorphism at IL-10 gene in humans. In this way we can proposed better drug in better dose according to genetic makeup [3-5]. Let's discuss various characteristics of HCV.

A. Phenotypic and Biochemical characteristics of HCV:
Hepatitis characterizes as muscle pain, fatigue, abdominal pain and nausea, black colored urine, weight loss, loss of appetite, hepatomegaly and splenomegaly [1]. In HCV different types of biochemical parameters changes occur as ALT level [6], level of bilirubin, albumin, prothrombin, aminotransferases and alkaline transferases in the liver increases [7]. The initial indication of having hepatitis is jaundice that means high deficiency of blood and weakness with high weight loss [8].

B. Causes of HCV infection
Normally, infection of hepatitis B does not depend on age but it prevails in those persons that have less resistance against diseases due to excess use of antibiotics, persons acquire AIDS, blood transfusion of infected patient to normal and through sex. Serum and blood plasma is the most rapid transmission vector of hepatitis. It causes cirrhosis and hepatocellular carcinoma [8]. In 2014, HCV outbreak was reported in Europe. Other sources of HCV transmission are sexual contact with infected person; breast feeding that is most common route of HCV spread from mother to infant. It is vertical mode of transmission. Cosmetic practices are also involved in HCV prevalence [9]. Reviewing nationwide, it was found that India and Egypt are major driver of HCV by medical exposure. World Health Organization has indicated that still 39 countries did not follow the precautionary measures to limit HCV infection through infected surgical instruments and blood transfusion. By using contaminated syringes 44% patients acquired disease while 44.7% of patients become infected from HCV through hemodialysis. There

are various reasons for acquiring the disease and one reason is the use of contaminated syringes. 22% of risk factors for determining HCV infection are still unknown [10-13]. HCV infection can be caused by a number of factors. Preventing HCV susceptibility is extremely difficult. However, genetics, which is an ever-evolving science, can now solve this dilemma. On the basis of polymorphisms in genes involved in viral protection, genome analysis will expose a person's susceptibility to HCV infection [14].

C. Genetic characterization of HCV

Hepatitis C is caused by hepatitis C virus known as HCV. HCV belongs to flaviviradae RNA virus family. It consists of 40-50nm diameter. HCV is 3rd internationally and 4th in Pakistan among most prevailing diseases [10]. HCV genome consists of 7-9.5 kb having 5', 3' non coding regions (NCRs), structural and functional proteins. 5' NCR is highly conserved region while 3' NCR is short variable region. Structural proteins involve in production of core and envelop of viral particle. These proteins are named as E1 and E2. They are also involved in viral replication. Through glycosylation E1 and E2 secrete viral envelop. HCV genome consists of various domains; each domain is responsible for specific function. These domains are: helicase, serine, serine protease, serine protease cofactor, RNA dependent RNA polymerase (RdRp) as shown in the fig. 1 [15, 16]. HCV RdRp lacks proof reading; responsible for broad range of diversity of HCV genotypes.

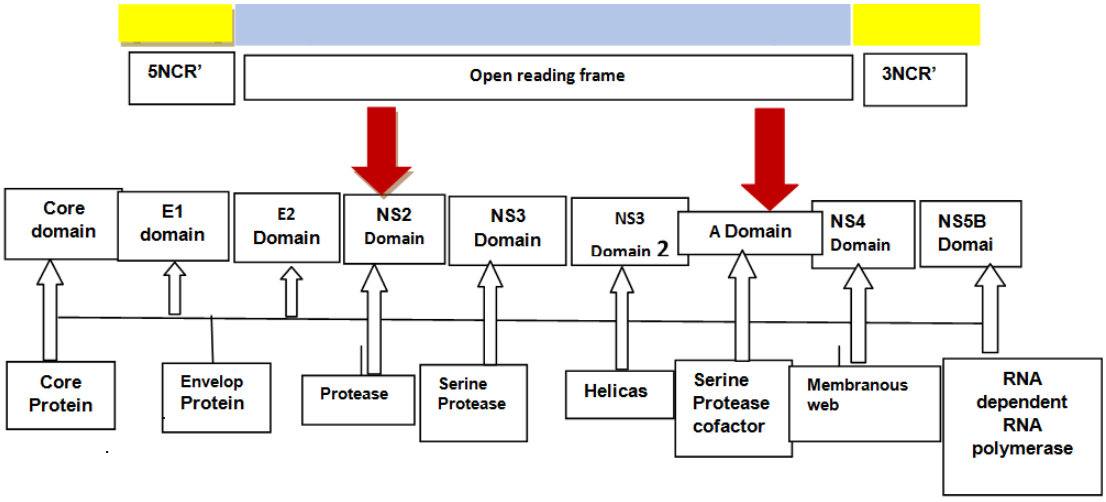


Fig. 1. Genetic characterization of HCV through description of various domain located on HCV genome [17, 18].

D. HCV replication cycles

Hepatitis C virus produces severe infection in patients but some have immunity against HCV naturally. When hepatitis virus enter into blood stream of any person despite of type of vector through which it spread or enter into human body; directs towards cellular nucleus and overcome biosynthetic machinery of the host and command it to produces thousands of copies of it and as a result of which cell bursts. Thus, infects other cells in the same way. This mechanism of action of virus is known as lysis or lysogenic cycle. The two types of cycles that determine replication of virus in a cell are lysogenic cycle and lytic cycle. Such a type of cycle for viral replication; in which lysis of cell occur but genetic material remain intact while only biochemical machinery affected, known as lysogenic cycles. Such a type of cycle for viral replication; in which lysis of cell does not occur but incorporation of foreign genetic material of virus occurs into DNA of host cell known as lytic cycle [19, 20].

E. Immune responses against HCV infection

It was considered that HCV have four kinds of genotypes base on viral kinetics and induction of immune responses in host; named as HCV1, HCV2, HCV3 and HCV4. HCV1 and 4 are involved in rapid viral response (RVR), while HCV2 and 3 are involve in sustained viral response **(SVR**) [21]. As HCV produces viral infection, it suppresses immunity of the host; while host body tries to compete with these infections. Potentiality of these immune responses against HCV infection also depends upon genotype of the host. But recent research reveals that

HCV has diverse group of genotypes including almost seven genotypes and 67 more their subtypes. HCV genotype 7 has been discovered but not studied well till now [22, 23].

II. MATERIALS AND METHODS

A detailed literature search was obtained using various online search engines like Science Direct, Pub Med, Google, Google Scholar, Web proof Science, Science Direct, EBSCO and SCOPUS.

A. Commonly distribution of HCV genotypes internationally

There are six commonly found genotypes of hepatitis C virus. Each of which is further subdivided into its subtypes on base of few minor genetic variations. Genotype 1 includes two subtypes named as HCV1a and HCV1b.this type of genotype is commonly found in developed areas like U.S.A, Central Asia and Europe. Genotype 2 includes four subtypes known as HCV2a, HCV2b, HCV2c and HCV2d. It is commonly found in

West Africa. Genotype 3 have 6 more subtypes named as HCV3a, HCV3b, HCV3c, HCV3d, HCV3e and HCV3f. It is commonly found in Southeast Asia; while genotype 4 is the most diverse class of HCV. It includes 10 subtypes alphabetically named as HCV4a, HCV4b, HCV4c, HCV4d, HCV4e, HCV4f, HCV4g, HCV4h, HCV4i and HCV4j. It is commonly found in Central Africa. Genotype 5 and 6 both have just one subtype known as HCV5a and HCV6a respectively. HCV5 is commonly found in South Africa and South Asia while in Southeast Asia, HCV 6 commonly occurs [16, 24].

Table 1: Commonly distribution of HCV genotypes internationally [25, 26].

HCV genotypes	HCV genotype subtypes	Commonly found in areas
HCV 1	2	U.S.A, Central Asia and Europe
HCV 2	4	West Africa
HCV 3	6	Southeast Asia
HCV 4	10	Central Africa
HCV 5	1	South Africa, South Asia
HCV 6	1	Southeast Asia

B. Distribution of HCV genotypes in Pakistan

It is observed that about 6% of Pakistani population is infected from HCV and there lies a comprehensive data that provide information about the presence of specific genotype of HCV in a specific area of population in Pakistan. In Pakistan, genotype 3 is the most prevailing form of HCV. It spreads in about 69.1% of HCV patients in Pakistan. On the other hand, HCV genotype 5 and 6 are found in the least ratio of 0.2% each. Genotype2 and 4 are found in 4.2% and 2.25 of Pakistani HCV population. By summarizing the data about distribution of HCV genotypes in Pakistan at province level we came into know that genotype 2 and 3 are most prevailing types of HCV. In KPK and Sindh, 17.3% and 11.3 % of HCV2 found respectively. While in Punjab, Sindh and KPK, 67.7%, 53.9% and 46.9% of HCV3 found respectively [27, 28].

C. Role of cytokines against HCV infection

Agents that are produced by the body as immune responses are known as cytokines. Cytokines belong to two classes of immune systems (Th1: T Helper Cell Type 1 and Th2: T Helper Cell Type 2). Mainly Th1 cytokines produce toxic effects while Th2 cytokines involve in immunity against infection. They act as anti-viral agents. These cytokines are protein in nature and encoded by genes. Level of production of cytokines in an individual is determined by type of polymorphism in genome of the host. Studies reveal that IL-10, 1L28B, and LT-A play role in HCV clearance [30]. Actually, an important cytokine known as IL-10 (interleukin 10) have anti-inflammatory action [31]. It belongs to helper T cells type 2 system, can be used in anti-viral therapy. During various infections, immune system excessively activates and leads to tissue damage in host. To control these immune responses, immunoregulatory agents known as cytokines play their role [32]. One of the major cytokines is known as interleukin-10(IL-10) [33-35]. T cell response by MHC at early stage of viral invagination is an important factor to activate host defense mechanism. In case of person who is most susceptible to HCV disease do not contain the genes responsible to functionalize MHC to detect virus [36] (Fig. 2).

D. Role of IL-10 in HCV clearance

IL-10 is homodimer in structure; having a molecular structure of 37kDa. Its main biological functions are anti-inflammatory and anti-aging agent. It is more important to maintain its concentration in blood serum because its low concentration leads to viral infection while high concentration leads to formation of cancerous lesions. It appropriate level in body is necessary in immune system. In its absence, immunity collapse down (Wilson, 2010 #31).

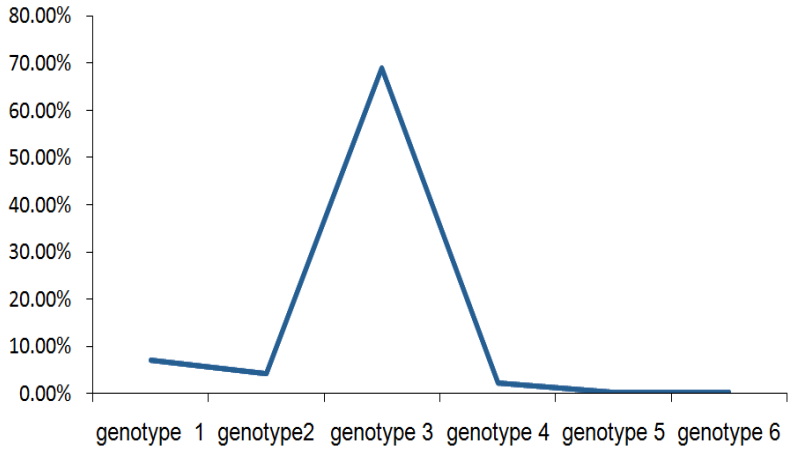
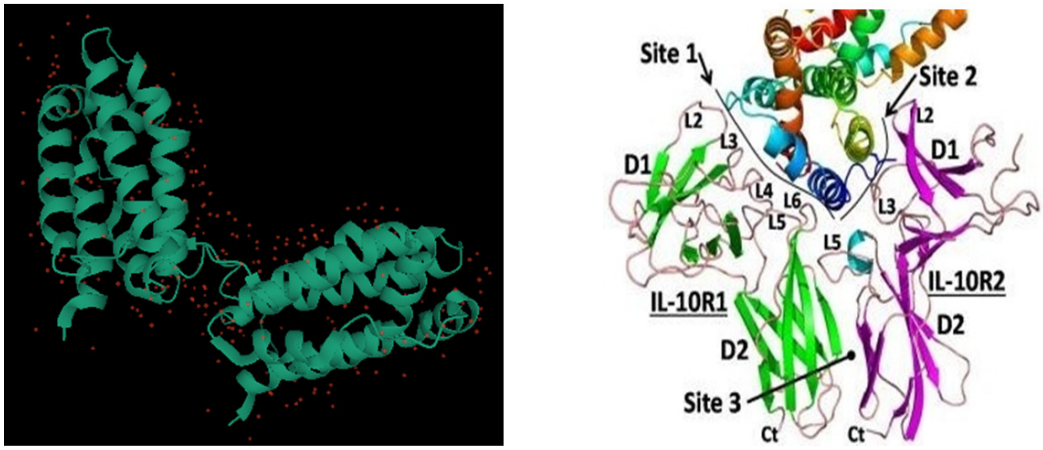


Fig. 2. Frequency of HCV genotype distribution in Pakistan [29].



(a) (b)

Fig. 3 (a) 3D structure of IL-10 (Figure adapted from ebi.ac.uk) **(b)** IL-10R1 and IL-10R2 complex (Figure adapted from ncbi.nlm.nih.gov).

It binds to a specific receptor known as interleukin-10 receptor (IL-10R). It is dimeric in composition; consists of two subunits named as IL-10R1 and IL-10R2. It predominantly expresses on leucocytes. It is highly unique in IL-10 recognition. It has binding affinity to ligand. While other IL-10R2 subunit is significant is recognizing a series of other cytokines secreted by IL-10. This set of cytokines secreted by IL-10 is known as IL-10 cytokines family. This family includes IL-22, 26, 28A, 28B and 29 [37-39].

When IL-10 binds with IL-10 receptor, it activates Jack STAT signaling pathway. As a result of which IL-10 enables to effect immune cells. IL-10 has broad spectrum of activity on leucocytes. They are important in inhibition of inflammation. Severe immunopathological effects occur; when IL-10 pathway is blocked. IL-10 is responsible for producing a range of immune cells. These cells include CD4, CD8, natural killer cells (NK cells), B cells, dendritic cells and macrophages.

IL-10 is therapeutic in action when delivered to targeted localization. By studying autoimmune diseases in IL-10 deficient mice reveals the importance of IL-10 in various critical functions. Studies show that IL-10 is important in maintaining homeostasis[40]. It is also responsible to stabilize the population of microbial flora in gut of stomach and lumen of intestine [5]. Deficiency of IL-10 produces colitis. Macrophages need IL-10 for their anti-inflammatory action. Natural killer cells control viral infections. Production and replication of NK cells is promoted by IL-10 [41-43]. IL-10 gene is highly polymorphic. It consists of about 5.2kb of nucleotide bases; having five exons on its length. It contains about ten number of polymorphism along the gene size. Seven types of polymorphism found on flanking region of IL-10 gene and three were present on the coding region of the gene. IL-10 gene is a member of chromosome 1 of human genome [44, 45].

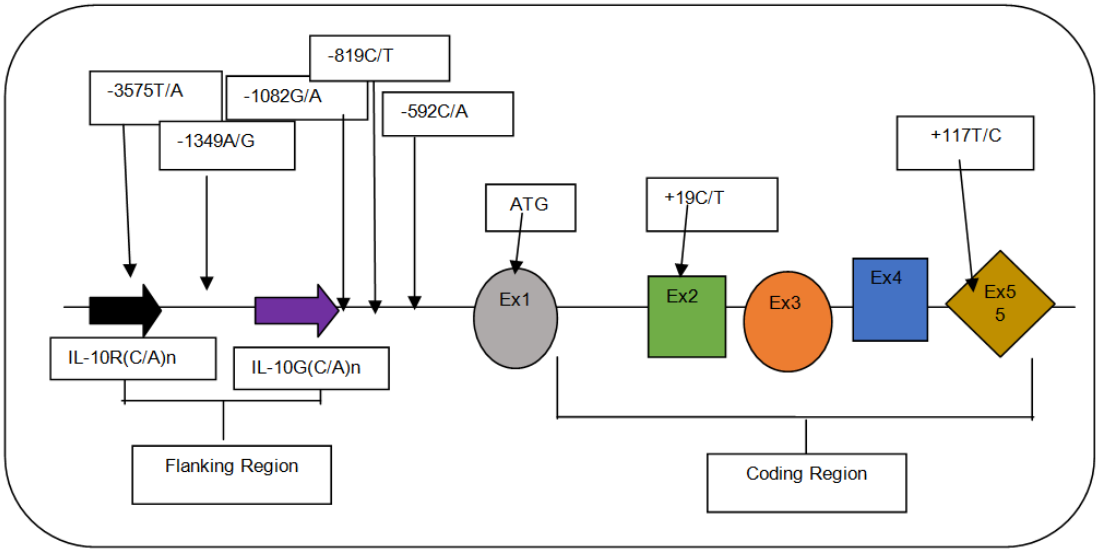


Fig. 4. Genome mapping of human IL-10 gene on base of different polymorphism [46].

E. Mutation spectrum of IL-10 in human population

In human population, a broad range of polymorphism occurred at promoter region of IL-10 coding gene. IL-10 gene is highly polymorphic in human population at -1082, -819, -592 region of promoter). These sites code for AAC, ATT and GCC repeats associated with mediate, low and elevated level of expression of IL-10 respectively. Among 60-80% of HCV patients that have HCV antibodies in blood serum, 15% of patients have immunity against it. Strength of immunity against HCV in host depends upon genetic makeup that will determine how person will response to HCV [47, 48]. INFg belongs to Th-2 system which induces immune responses [41]. It is also polymorphic (A/T) in nature at upstream region of 874 on promoter. 874T expresses high level of INFg; as compare to A-type polymorphism. Rate of expression of INFg follows TT>TA>AA order. Mutation at genes coding CD4+ and CD25 decreases secretion of IL-10 by Th2 system become persons susceptible to HCV infection. Patients suffering from severe infection having homozygous mutation instead of heterozygous mutation [2]. During severe HCV infection, three types of polymorphism have been observed at 1082, 819 and 592 in form of G/A, T/C and A/C respectively; expresses broad range of level of IL-10. Its promoter site is surrounded by three kinds of microsatellite repeats like GCC, ACC and ATA [49, 50].

III. CASE STUDY

HCV was identified by simple device method. Those devices that shown positive test further used for ELISA. HCV could be diagnosed by various techniques such as ELISA, PCR and recombinant immunoblot assay. ELISA is known as enzyme linked immunosorbent assay. Real Time-Polymerase Chain Reaction was used to quantify RNA in patient samples [51-53]. By summarizing the data of different populations we observed patients and controls in order to compare occurrence of types of polymorphism among genes of IL-10and its linkage with susceptibility to acquire HCV in human population. We just studied polymorphism at IL-10 because its genes are directly linked to produce immunity against viruses. Hence they are important in viral clearance during infection [54].

In case of 819 region of IL-10 gene, C/C SNP was common in normal person as compare to HCV patients that have C/T or T/T SNP [55]. Without treatment 15-45% can recover within 6 months of infection. Treated HCV infection cannot immune person against HCV infection in future. Prevalence of HCV depends upon person socio-economic condition, immunity, PWID (person who inject drug) and its genotype especially on base of IL-10 polymorphism. DAA therapy stands for direct acting antiviral therapy. It is oral type of therapy that is most effective strategy to control HCV infection [56].

In patients level of IL-10 was found to be decreasing instead of controls. Level of IL-10 production depends upon polymorphism exists in IL-10 gene. -1082GG, -819CC and -592CC are involves in highest concentration in the body. By summarizing the data from all populations, we observed that GCC is most common in normal persons as compare to the infected population [57].

To determine the distribution of different IL-10 alleles among Pakistani population 89 patients and 99 controls were observed for study. It was found that GCC/ATA is the most common Haplotype among HCV patients; while GTA, ACC, GCC/GTA and GCC/ATA haplotypes were present in frequency of 6.9/3.30, 7.3/2.02, 12.4/2.02 and 55.1/75.76% among Pakistani population respectively (Fig. 5&6).

-1082G/A	-819C/A	-592C/A	Haplotype/diplotype
GG	CC	CC	GCC GCC/GCC
GA	CT	CA	ACC GCC/ACC GCC/ATA
AA	TT	AA	ATA ACC/ACC ACC/ATA ATA/ATA

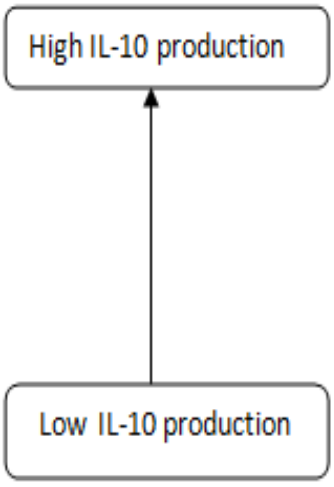


Fig. 5. Interrelationship between IL-10 production and type of polymorphism present in an individual [37].

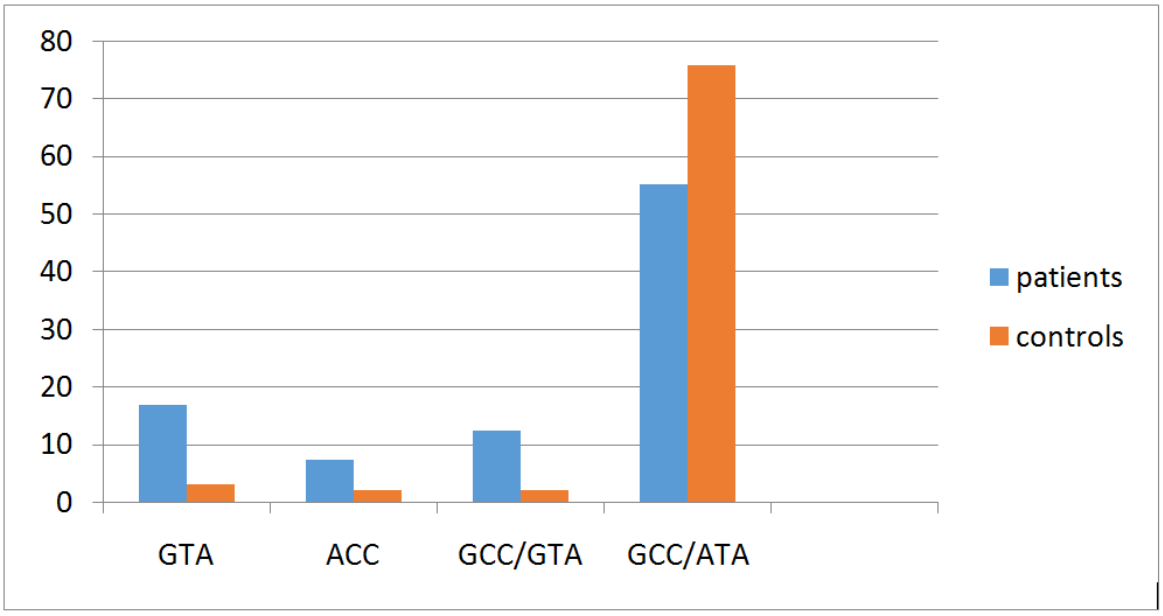


Fig. 6. Distribution of different IL-10 alleles among Pakistani population [58].

IV. CONCLUSION

Hepatitis C is a devastating viral disease that affects liver and eventually causes death of that person. During this disease, level of IL-10 reaches to the lowest level. IL-10 plays an important role in regulating immune responses. It is responsible for viral and bacterial clearance from the body. The strength of immune system directly linked with appropriate level of IL-10 in the body. IL-10 is secreted by Th2 system. Level of its production correlate with type of polymorphism located at IL-10 gene on chromosome no.1 in homo sapiens. IL-10 is highly polymorphic gene. This polymorphism is responsible for susceptibility to HCV when secretes IL-10 in low concentration. On the other hand, if polymorphism supports proper production of IL-10 provide immunity against viral infections. In this article, we can conclude that natural immunity against viruses could be attained by increasing IL-10 production in the body. In case of HCV treatment should be done according to genotype of patient. Low IL-10 producing genotyped patient should be given a high dose of cytokines. While high IL-10 producing genotyped patient should be given a low dose of cytokines.

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Conflicts of Interest: The authors declare no conflict of interest.

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